Medial Dorsal Thalamic Lesions and Working Memory in the Rat

Thesis

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MEDIAL DORSAL THALAMIC LESIONS AND WORKING MEMORY IN THE RAT

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ABSTRACT

Pigmented rats of the DA strain with either radiofrequency or ibotenic acid lesions of the thalamic nucleus medialis dorsalis were postoperatively given non-spatial and spatial tests of working memory. In the non-spatial task, delayed non-matching to sample, rats with both types of thalamic lesions showed acquisition impairments. The subgroup of rats with nucleus medialis dorsalis lesions that were able to reach the acquisition criterion did, however, perform normally when the retention interval was extended to 60 seconds and when proactive interference was increased. A final, control variant of this task was included to address the possibility of a sensory deficit. In the spatial task, delayed forced alternation, rats were tested with differing retention intervals and with both spaced and massed trials. Damage to nucleus medialis dorsalis had no effect on acquisition or on spaced trials, but a slight deficit was found in the animals with radiofrequency lesions under the massed trial condition. Much clearer deficits were, however, present in those animals in which the lesion extended appreciably into the anterior thalamic nuclei. The findings indicate that while cellular damage to nucleus medialis dorsalis may disrupt learning, some impairments in tests of spatial working memory attributed to this nucleus may reflect damage to the adjacent anterior thalamic nuclei.

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CHAPTER ONE

INTRODUCTION

Memory has been described as "The ability to receive a sensory impression, to retain it and to recall it at the appropriate moment" (Brierley, 1977, p.199). This process of memorizing is, however, seemingly impossible to isolate as a defined system, being bound up with a confusion of other contributive and dependent processes ranging from perception to action.

In spite of this problem it does appear that a number of relatively well-defined structures are pre-eminently important for certain classes of memory. That is to say that they appear to be necessary for normal memory, but not for a wide range of other cognitive functions.

By the use of rats as a model of human diencephalic amnesia, this study will attempt to help clarify the role in memory played by the thalamic nucleus medialis dorsalis (MD), a structure which has often been implicated in studies of memory function.

1.1 Anatomical Properties and Connections of MD.

The thalamus is the dorsal part of the diencephalon; the other parts being the hypothalamus, subthalamus, and epithalamus. The brains of all mammalian species include a thalamus, or more correctly two thalami, one in each hemisphere, connected through the third ventricle by the massa intermedia. The thalamic regions that have been implicated in memory processes are MD, the medial pulvinar, the laterodorsal nucleus, and the anterior nuclei. Of these MD is the most frequently associated with important memory function.

The location of MD is medial to the internal medullary lamina in the middle third of the thalamus. In primates MD can be readily divided into three regions on cytoarchitectonic grounds. These are the medial magnocellular portion (MDmc), the lateral parvocellular portion (MDpc), and the far lateral multiformis portion (MDmf) (Olszewski, 1952; Akert, 1964; Tobias, 1975; Goldman-Rakic & Porrino, 1985). Olszewski (1952) also refered to a small densocellular portion in the most caudal part of the nucleus. Although MD in the rat is much more homogeneous in its cytoarchitectonic structure, fibre preparations have helped distinguish a medial, a central and a lateral region (Cornwall & Phillipson, 1988; Krettek & Price, 1979; Leonard, 1969), and Groenewegen (1988) has added a paralamellar area in the extreme lateral region.

In order to establish an analogy between MD in rats and primates (including humans), it is necessary to compare these divisions on the basis of their projections. Thus, in the far lateral part of MD, the rat's paralamellar segment would appear to correspond with the pars multiformis in primates; both having substantial reciprocal connections with the frontal eye fields (Groenewegen, 1988; Olszewski, 1952). This then leaves the apparent anomaly of the rat having three remaining segments (medial, central, and lateral) to compare with only two in primates (magnocellular and parvocellular). This anomaly may be resolved by the discovery that the central portion of MD in the rat has heavy inputs from olfactory regions such as the ventral orbital cortex, prepiriform cortex, and olfactory tubercle (Groenewegen, 1988). Similar rhinencephalic afferents in the primate terminate in MDmc (Russchen, Amaral, & Price, 1987) (fig.1). Given that the rat has evolved to rely heavily on olfaction, it seems reasonable to regard this central portion as a relatively specialised part of what would be MDmc. Other evidence indicating that the combined central and medial portions of MD in the rat correspond to MDmc in the monkey comes from the finding that in both orders it is these regions of MD that are connected with the ventral and orbital portions of the prefrontal cortex and receive limbic inputs from structures such as the amygdala and entorhinal cortex

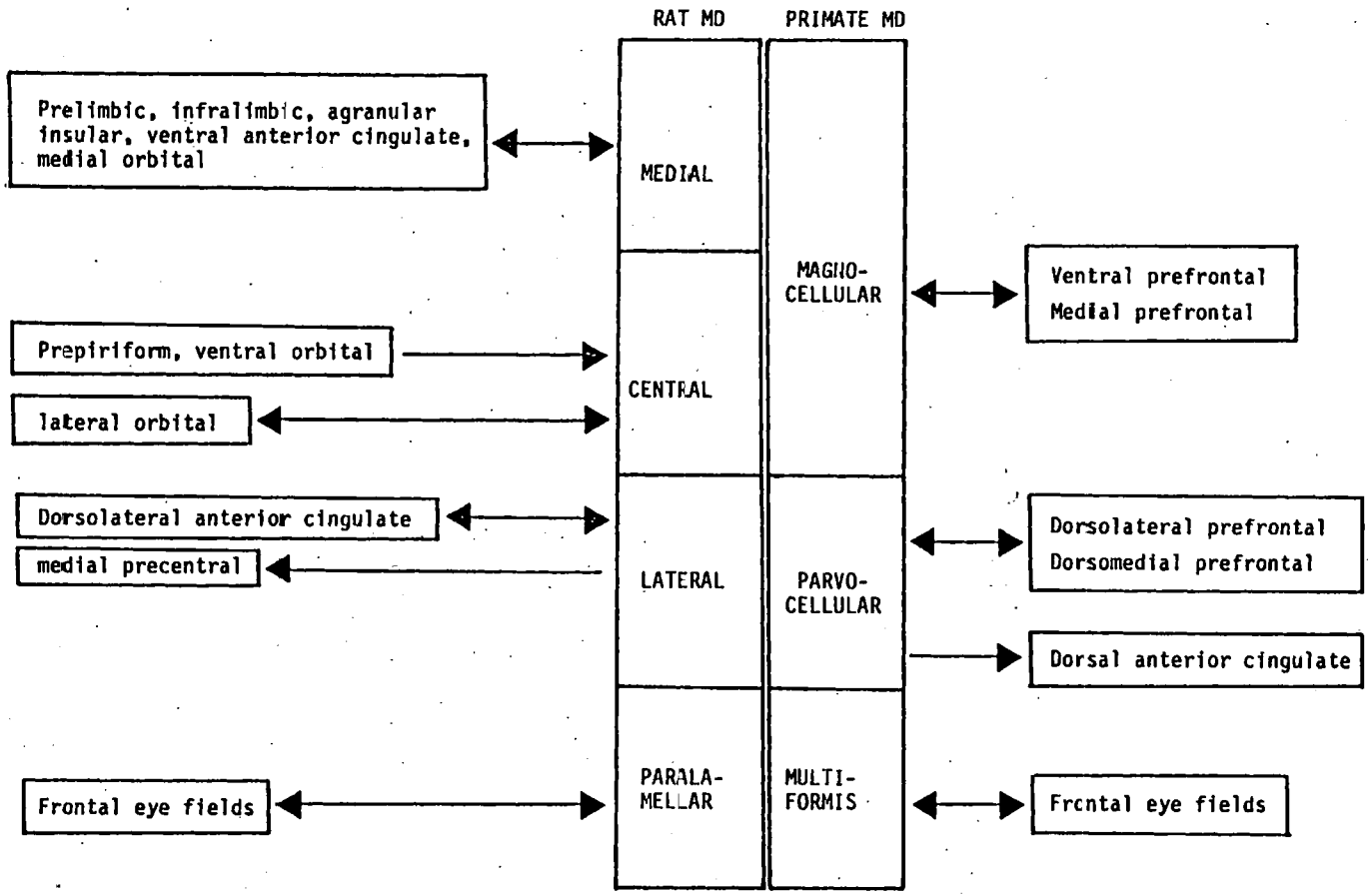

Fig. 1. Frontal cortical connections of MD.

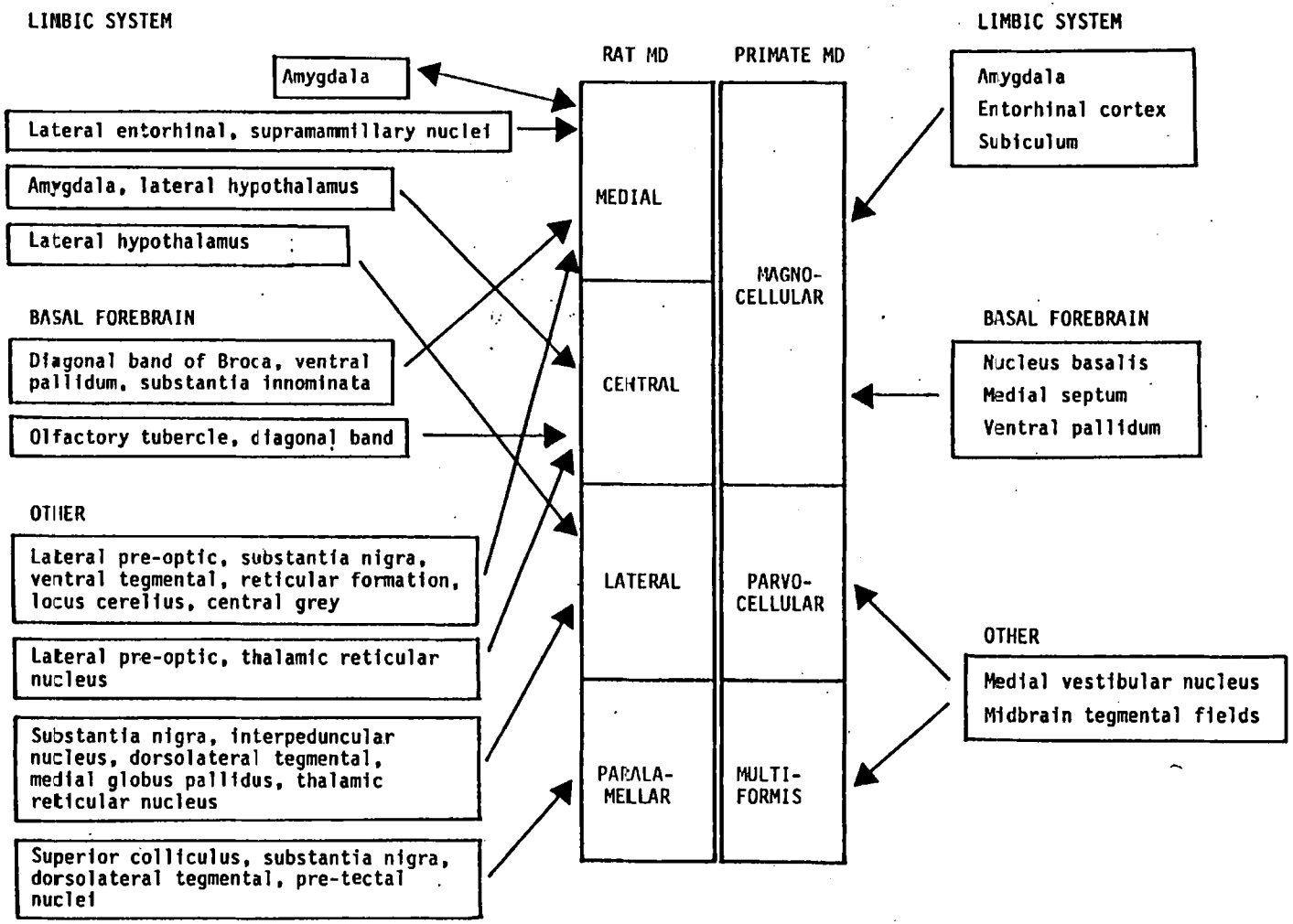

Fig. 2. Subcortical connections of MD.

(Cornwall & Phillipson, 1988; Groenewegen, 1988; Nauta, 1961; Russchen et al, 1987). Similary, both the lateral MD in the rat and MDpc in the monkey are connected with the dorsal and lateral prefrontal cortex (Fig. 1). Thus it can be seen that the divisions of MD are comparable in rats and primates; the central and medial portions in rats being analogous with MDmc, and likewise the lateral portion being analogous with MDpc.

The major anatomical connections of MD in the rat, which are illustrated in Figures 1 and 2, can be divided into four groups. First, there are limbic system structures, such as the amygdala and lateral hypothalamus. These connect largely with the medial and central portions of MD. Second, there are basal forebrain structures, such as the ventral pallidum and olfactory tubercle , which also connect with the medial and central portions. Third, there are areas of the frontal cortex, such as the prelimbic and medial precentral, which connect with the medial, central, and lateral portions. The frontal eye fields are an exception to this, having reciprocal connections with the paralamellar portion of MD. The remaining major connections which do not fit into these three groups include such structures as the substantia nigra and thalamic reticular nucleus, and these connect diffusely throughout MD.

An examination of the connections of MD in the monkey (Figs. 1 and 2) reveals a very similar picture to

that in the rat with, once again, the major connections being with limbic, basal forebrain, and frontal cortical regions.

In summary, it can be seen that the rat MD bears many connectional similarities with the primate MD, and as a consequence may prove to have similar functions. This may lead to an experimental situation where suitably analogous memory tasks can be applied to rats, monkeys and humans with the ultimate aim of using the rat as a model for some aspects of human diencephalic amnesia.

1.2 Classifications of Memory Processes

Most workers in the field of memory have found it possible to recognize three types of memory in terms of duration, i.e. the brief sensory store (iconic or echoic memory), short-term memory, and long-term memory. Although the actual lengths of time ascribed to these terms vary widely, they cannot be regarded as merely relative terms, as the physiological mechanisms behind them can be differentiated.

Furthermore, a hierarchical structure can be seen in long-term memory, such that it comprises procedural memory (knowing "how") and declarative memory (knowing "that") (Tulving, 1985). Procedural memory is then subdivided into classical conditioning, motor skills, and priming;

declarative memory into episodic and semantic memory (Parkin, 1987; Tulving, 1985). Episodic memory is memory for autobiographical events as opposed to semantic memory which is memory for language, facts, general concepts and rules about the world.

Another important differentiation which has often been made, particularly in research involving animal memory, is that of 'working' memory and 'reference' memory (Honig, 1978; Olton, Becker, & Handelman, 1979). This distinction is made on the premise that the process of memorizing a general rule (reference memory) differs from the ability to memorize a piece of information necessary to complete a single subsequent task (working memory). Therefore, in an experimental context like this study, working memory would provide the information needed to perform a single trial correctly, and reference memory would provide the rule as to how to perform all such trials.

1.3 Diencephalic Amnesia and the Study of Memory

In order to attempt to relate memory functions to specific brain loci, much use has been made over the years of cases of memory loss. This can be found in amnesic syndromes in human clinical cases, or deliberately created by producing brain lesions at sensitive sites in animals. In clinical amnesic syndromes in humans, the cause is usually brain damage of some kind caused by disease, trauma,

or surgical intervention.

Amnesia is often divided into anterograde amnesia, the memory loss acting on material perceived after the onset of brain injury/degeneration, and retrograde amnesia, pertaining to material perceived before the onset. Although both types of amnesia can be present in the same subject, anterograde amnesia has been of particular interest as the learning deficit occurs in the face of normal or near-normal performance in a wide range of other cognitive tasks. This points to the fact that certain brain structures can be regarded as being pre-eminently concerned with the encoding, storage, or retrieval of new memories.

Anterograde amnesia is usually divided into two classes, reflecting the two anatomical regions within the brain with which the amnesia is associated. These regions are the temporal lobe (encompassing structures such as the hippocampus, fornix, and amygdala), and the diencephalon (including, amongst other structures, the mammillary bodies and several thalamic nuclei including MD). Therefore, as this series of animal experiments is based on MD, evidence from human diencephalic amnesia has been examined as a background to the work. As can be seen from the studies described below, however, there are considerable difficulties in obtaining accurate data from human cases, and this highlights the need for precise examination of the mnemonic effects of diencephalic lesions in animals.

Human cases of surgically-induced diencephalic amnesia are rare, and little knowledge has been gained from this source as compared with temporal lobe amnesia. It is, in any case, a highly undesirable by-product of neurosurgery and unlikely to be reproduced deliberately. Accidental brain-damage has supplied some data, but cases of circumscribed trauma lesions causing amnesia are rare. More information has been gained from lesions caused by disease processes which have led to amnesia, such as tumours and infarction. However, the greatest cause of diencephalic amnesia and also the best-studied is Korsakoff's syndrome. The amnesia caused by Korsakoff's syndrome is useful to the study of memory because, in many cases, it is relatively selective, leaving the intellect virtually intact.

1.4 Evidence from Cases of Human Diencephalic Amnesia

1.4.1 Korsakoff's Syndrome

Korsakoff's syndrome is a symptom-complex related to vitamin deficiency causing widespread degeneration of the brain. The thalamus and mammillary bodies are most commonly subject to some damage in Korsakoff's syndrome.

In 1887, Korsakoff, a nineteenth century Russian physician, first described this syndrome in cases of uterine infection, puerperal septicaemia, typhus, tuberculosis,

diabetes mellitus and cases of poisoning by arsenic, lead carbon monoxide and ergot. However, during his studies of the syndrome, most of his evidence came from causes of chronic alcoholism, which is still the case today.

Gudden, in 1896, linked Korsakoff's findings with the neuropathology of two chronic aloholics and one case of sulphuric acid poisoning described by Wernicke in 1881. The term "Wernicke-Korsakoff syndrome" is sometimes used because of this association, but generally Korsakoff's syndrome or psychosis is distinguished as being the chronically amnesic form of the syndrome. Korsakoff described patients with "a derangement of memory and of the association of ideas" (Victor and Yakovlev, 1955, p.396).

Korsakoff's syndrome has long been linked with thiamine (vitamin B1) deficiency which can cause a long-term inability to metabolise glucose, resulting in a distinctive, widespread brain-tissue degeneration. The sort of prolonged and severe thiamine deficiency required to produce this state is usually found in chronic alcoholics, whose general malnutrition combines with the reduced ability of the gut to absorb thiamine in the presence of alcohol.

That the syndrome is relatively common can be seen by hospitals in urban areas with large populations of alcoholics reporting diagnosis rates as high as one per thousand admissions (Kalat, 1984). Enlarged ventricles are found on pathological examinations in fifty to one hundred

per cent of alcoholics (Parsons and Prigatano, 1977).

The relationship between chronic alcohol intake, thiamine-deficiency and learning impairments has been demonstrated in rats by Irle and Markowitsch (1983b), and a Korsakoff-like anterograde amnesia has been induced in thiamine-deprived monkeys by Witt and Goldman-Rakic (1983a & b).

The anatomical and behavioural effects of the syndrome are, however, controversial. Memory impairments are the main symptom; other symptoms which are not memory-related being general inertness and apathy, and minor deficits in perceptual and conceptual functions. Victor, Adams, and Collins (1971) distinguish Korsakoff's psychosis as affecting memory disproportionately, whilst leaving other areas of brain functioning relatively clear.

Since the beginning of this century four 'cardinal elements' of Korsakoff's syndrome have been recognized in relation to memory deficits. In order of their relative frequency of occurrence these are anterograde amnesia, retrograde amnesia, disorientation in space and time, and confabulation.

The anterograde amnesia element was first noted by Korsakoff himself, and is the most interesting for the purposes of this study, being amenable to study in animals. However, retrograde amnesia does occur in Korsakoff subjects and in some cases the amnesia covers almost their entire

adult lives (Kalat, 1984).

Disorientation in space and time is a memory dysfunction sometimes noted in the syndrome and takes the form of an upsetting of the chronological order of events.

Confabulation, the tendency to invent or improvise events and to substitute them for gaps in memory, is also a feature sometimes found in Korsakoff's syndrome. Faulty recognition of people is also often involved in such confabulation.

Studies of Korsakoff subjects using tests analogous to animal studies of memory have shown impairments in both spatial (Oscar-Berman & Zola-Morgan, 1980; Oscar-Berman, Zola-Morgan, Oberg, & Bonner, 1982) and non-spatial tasks (Aggleton, Nicol, Huston, & Fairbairn, 1987; Oscar-Berman & Bonner, 1985 & 1989).

Precisely which brain structures are most critically affected in Korsakoff's syndrome is important to the study of memory and has been much researched. Damage to the mammillary bodies and the mediodorsal thalamic nuclei was noted in Korsakoff patients as long ago as 1896 by Gudden who described shrinkage, loss of cells, and gliosis at these locations. The relative importance of these structures, though much disputed over the years, remains unconcluded.

Traditionally, the assumption has been that mammillary body damage is the pre-eminently important factor in the syndrome, based on a number of relatively early

studies which stress their importance (e.g. Benedek and Juba, 1944; Delay, Brion, & Elissalde, 1958a; Gruner, 1956; Malamud and Skillicorn, 1956; Orthner, 1957; Remy, 1942). However, these studies could be criticised in that they tended to base their observations of mammillary body damage on gross pathological changes visible to the naked eye, which could be misleading. This is illustrated by Delay et al's (1958a) description of mammillary bodies appearing atrophic and yellow, yet on microscopic analysis they found loss of neurones to be only slight.

Cravioto, Korein, and Silberman (1961) stress the importance of the mammillary bodies in their study of 28 cases, all of which showed bilateral mammillary body changes, and half of which were gross lesions. However, of the 22 brains which were examined microscopically, consistent thalamic damage was found in all of them, particularly in the anterior nuclear group.

The large-scale and authoratative study of Victor et al (1971) also found little evidence of gross pathalogical lesions in the thalamus, but mammillary body lesions were apparent to the naked eye in 74 per cent of cases. However, despite these gross pathological findings, their study provides the most widely accepted evidence for the predominant involvement of the thalamus and particularly the mediodorsal nucleus. Over 80 per cent of the Korsakoff patients examined post-mortem showed changes in the thalamus

when this was examined microscopically. Of these cases, 88.4 per cent had lesions of the mediodorsal nucleus, principally in the magnocellular portion, and 85 per cent lesions of the medial pulvinar nucleus. The authors noted the ambiguous boundary between these two nuclei, but implied that the lesions of the latter constituted an extension of those of the former. In fact, MD was affected in every case of thalamic involvement, and in 20 per cent of these cases it was the only thalamic nucleus found to be involved.

Although the authors did not claim to decide with certainty which structure was pre-eminently critical in memory function between the mammillary bodies, the mediodorsal nucleus, and the medial pulvinar nucleus, they did provide strong evidence in favour of the mediodorsal nucleus as follows.

Of the 43 brains examined post-mortem in the study, only five were found with no change in the mediodorsal nuclei. These were also the only five cases which in life had shown no memory defect but only symptoms of Wernicke's disease. The mammillary bodies were affected in all five cases. The authors conclude that the mammillary bodies may be significantly affected in cases where there is no memory defect. They sum up by stating their belief that the amnesic defect is related to lesions in the diencephalon, specifically in the medial dorsal nuclei and possibly in the medial pulvinar.

It seems reasonable then to conclude that within the considerable experimental constraints of human studies, anatomical examinations of the brains of Korsakoff patients can indicate a plausible link between memory function and the thalamus, and within the thalamus most strongly the mediodorsal nucleus.

1.4.2 Other Thalamic Damage

1.4.2.1 Tumours

The disturbances of memory, often described as Korsakoff-like symptoms, associated with tumourous growths in the diencephalon have been used to provide evidence of the link between memory and diencephalic structures. It is, however, generally noted that the effects of such growths are diffuse and cannot be described with anything like the precision of those caused by other kinds of damage, e.g. ischemic damage. It is also recognised that tumours can cause damage distal from their site due to the surrounding oedema they create. (McEntee, Biber, Perl, and Benson, 1976)

According to Markowitsch and Pritzel (1985) tumours of the dorsal midline structures of the thalamus are frequently found to be the cause of memory disturbances; their removal sometimes resulting in complete recovery of

memory function.

A bilateral thalamic tumour with no mammillary body involvement, but unfortunately no specific description of thalamic structures involved, was reported by Sprofkin and Sciarra (1952) to be associated with amnesic symptoms.

McEntee et al (1976) described a case of amnesia associated with bilateral tumour invasion of the medial and posterior thalamus, including the mediodorsal nucleus without concomitant involvement of the mammillary bodies or anterior thalamus.

Tumours on the floor and/or walls of the third ventricle have long been implicated in memory disturbance syndromes. Weisenburg, in 1911, reviewed a number of cases of memory disorders and concluded that the third ventricle tumour was causing increased pressure on the cerebral cortex, rather than the damage being done directly to diencephalic structures (Brierley, 1977).

Williams and Pennybacker (1954) found that third ventricle tumours had resulted in amnesia due to increased pressure on diencephalic structures. Although it is often assumed that the structures most affected by this pressure are the mammillary bodies, the nature of ventricular pressure does not appear to lend itself to such differentiation, and other structures bordering on the ventricle may be similarly implicated, including the thalamus.

The surgical removal of tumours of the diencephalon in cases showing amnesic symptoms has produced effects on subsequent memory performance varying from complete restitution to a worsening of memory functions (Foerster and Gagel, 1934; Geffen et al, 1980). Williams and Pennybacker (1954) found that aspiration of fluid from the third ventricle as well as tumour removal resulted in improvement in memory defects, but the report of this is poorly documented.

1.4.2.2 Circulatory Disturbances

After damage caused by chronic alcoholism, the most frequent cause of diencephalic damage is disturbances in blood circulation (Markowitsch and Pritzel, 1985). Occlusion or rupture of major arteries can disturb or prevent normal metabolism in the pertinent brain area, often causing widespread and enduring amnesic states. In the case of thalamic damage, the relevant vessels would be the paramedian thalamic arteries.

Bilateral infarcts caused by arterial occlusion in the thalamus are usually accompanied by mnemonic disturbances. In one case a unilateral infarction of the thalamus showed signs of only verbal memory deficit (Michel, Laurent, Foyatier, Blanc, and Portafaix, 1982).

Computer tomography scans (eg Speedie & Heilman, 1982; Winocur, Oxbury, Roberts, Agnetti, & Davis, 1984) and

more recently magnetic resonance imaging techniques (Bogousslavsky et al 1988) have allowed such damage to the thalamic nuclei to be identified more accurately These are cases of restricted medial thalamic damage of ischemic origin associated with memory disturbances, and were both unilateral and bilateral.

The studies involving cases of unilateral damage failed to agree on the question of lateralization, with only some finding evidence of a link between verbal memory and damage to the left thalamus. One case (Speedie and Heilman, 1982), who had a discrete lesion of the left mediodorsal nucleus, had an anterograde memory impairment for verbal material. This case was compared with that of N.A., the patient with a stab wound in the same location (Squire & Moore, 1979). Behavioural tests showed both cases to be both quantitively and qualitatively similar.

1.4.2.3 Trauma

Thalamic damage sustained in accidents can have an effect on memory; memory disturbances being the most frequently reported behavioural consequence of traumatic brain lesions. Retrograde amnesia is more common in trauma than damage caused by tumours or circulatory disturbances, and a number of cases have been described where head injuries have caused symptoms paralleling those of Korsakoff patients. (Markowitsch and Pritzel, 1985.) However, the

nature of most head injuries makes the correlation of memory functions with a relatively circumscribed lesion difficult (Brierley, 1977). A well-known exception to this is case N.A.

In 1960, at the age of 22, this much-studied patient suffered apparently very localized diecephalic damage as a result of a mishap with a miniature fencing foil. The location of the lesion was identified in 1978 by computer tomography scan to be in the mediodorsal region of the left thalamus (Squire & Moore, 1979).

The resulting anterograde amnesia is markedly worse for verbal than for non-verbal material, although his intellect remains high with an IQ of 124. In fact, he has achieved higher scores than control subjects in many perceptual and cognitive tasks (Squire and Zola-Morgan, 1983). His amnesia differs from that of Korsakoff patients in that there is almost no retrograde amnesia, no impairment in tasks involving the temporal order of recent events, and he is able to exhibit release from proactive interference. (Markowitsch and Pritzel, 1985.)

However, a more recent study (Squire, Amaral, Zola-Morgan, Kritchevsky, & Press, 1987) using magnetic resonance imaging showed evidence of damage to areas outside MD, including notably the mammillary bodies. This finding highlights the difficulty of using much of the human evidence which is available.

1.4.2.4 Surgically Induced Lesions

Although there has been a significant number of studies of surgical thalamic lesions performed on human subjects for clinical reasons, information on the mnemonic effects of these interventions is rare. There are several reasons for creating such lesions, e.g. pain-relief, control of aggressive behaviour, control of epilepsy (Markowitsch and Pritzel, 1985). As these are attempts to modify the emotional behaviour of individuals, the post-operative effects on memory are not the primary behavioural study. Furthermore, the fact must be noted that such surgery is confined to patients whose abnormal behaviour has been attributed to abnormal brain activity. This makes interpretation of any data thus gained difficult to apply to any general theory of thalamic memory function.

Of those studies which have noted amnesic effects after lesions of MD, Spiegel, Wycis, Orchinik, and Freed (1955, 1956) provide a little evidence of a similarity to Korsakoff's syndrome. They describe a temporal disorientation (chronotaraxis) similar in some respects to Korsakoff's syndrome, but differing significantly in its duration. The symptom lasted generally for a few days or weeks with only one case lasting for six months. The location of these lesions also remains to be confirmed.

There is even less evidence for the involvement of

the anterior nuclear group from human lesion cases. Brierley (1977) states his opinion that no such lesions, either surgical or tumourous, have been shown to correlate with a true amnesia. Markowitsch and Pritzel (1985) suggest, however, that lasting impairments are found when lesions are made in patients with other existing damage to the central nervous system e.g. Parkinson's disease.

1.5 Animal Evidence

Because of the difficulties and limitations of using purely human data for the study of memory systems, animals, and especially non-human mammals, have been used in the attempt to develop models for human amnesic syndromes. As human studies of memory have made great use of memory losses and deficits to infer the existence and morphology of memory mechanisms, animal studies have been needed to establish the necessary and sufficient conditions under which the clinical syndromes occur. In this way the memory systems and inter-relationships of anatomical structures apparent in man may be paralleled, tested and possibly confirmed.

The main limitation of using human amnesic patients in the study of memory is that, for obvious ethical reasons, the theraputic benefit to the individual always has to take precedence over the gaining of useful data, even though this

may assist in the understanding of the overall problem and ultimately be more useful in future therapy (Olton, 1985). Animals are therefore used to gain precise knowledge relatively rapidly.

Other difficulties encountered in human studies include the size of sample groups available to the experimenter, the necessarily long delay in obtaining pathological data, the relatively uncircumscribed nature of non-deliberately induced lesions, and the interference of other factors in clinical subjects.

Although invertebrates are often used to study neural mechanisms at the cellular level, and to build up 'phylogenetic trends' (Markowitsch and Pritzel, 1985) in order to infer the human situation, non-human mammals are almost exclusively used to produce models of human memory.

Despite the long history of memory studies using animals, it is only quite recently that reliable and appropriate methods of memory testing have been developed which enable animal models to yield the kind of valuable knowledge which they now provide (Squire & Zola-Morgan, 1985).

Regarding the controversy of the relative importance of thalamic nuclei versus mammillary bodies, Victor et al (1971) reviewed some of the animal experimental literature. After pointing out the difficulty of directly equating animal behaviour after brain lesions with human behaviour in

Korsakoff's syndrome, they cautiously suggest that animal studies do bear out their conclusion in humans that the thalamic nuclei, especially the mediodorsal, are of primary importance. They go on to point out that mammillary body lesions produce very variable behavioural results depending on the type of memory task used, and that lesions of the thalamic nuclei must be bilateral and virtually complete in animals. This latter point is also made by Squire and Zola-Morgan (1983), and Stokes and Best (1990c).

The finding that aspiration or radiofrequency lesions of MD in the monkey can disrupt tasks such as delayed response (Isseroff, Rosvold, Galkin, & Goldman-Rakic, 1982), delayed nonmatching-to-sample (Aggleton & Mishkin 1983a; Aggleton & Mishkin, 1983b; Zola-Morgan & Squire 1985) and delayed alternation (Isseroff et al., 1982) has been interpreted as consistent with the supposed contribution of MD damage to human diencephalic amnesia.

A larger number of studies have used rats to measure the effects of lesions in MD on tests of learning and memory. The effects of conventional (radio frequency/electrolytic) lesions have, however, been inconsistent. Significant impairments have been reported for the re-acquisition of the radial arm maze task (Stokes & Best, 1988), spontaneous spatial alternation (Weiss & Means, 1980), reinforced spatial alternation (Vicedomini,

Corwin, & Nonneman, 1982) and go/no-go alternation (Winocur, 1985), while less clear-cut impairments have also been reported for spatial alternation (Brito, Thomas, Davis, & Gingold, 1982) and spatial reversal tasks (Means, Hershey, Waterhouse, & Lane, 1975). In contrast, other studies have provided evidence that MD lesions do not affect performance on tasks such as spatial alternation (Greene & Naranjo, 1986; Tigner, 1974), the Morris water maze, and the radial arm maze (Kolb, Pittman, Sutherland, & Whishaw, 1982).

One problem in the interpretation of these conflicting results is that conventional surgical techniques damage fibres of passage as well as neurons located in the nucleus. This problem is of particular concern for a subcortical nucleus like MD which not only contains many fibres of passage but is also bounded by a major fibre pathway, the internal medullary lamina. It is therefore possible that small differences in lesion locus and extent could have marked behavioural consequences. For these reasons the outcome of the few studies that have used neurotoxins which help spare fibres of passage (Scwarcz, Hokfelt, Fuxe, Jonsson, Goldstein, & Terenius, 1979) may be of particular interest. Unfortunately such studies have so far also proved inconsistent, with reports of normal performance on spatial reversals, the radial-arm maze, and go/no-go alternation in a straight alley (Beracochea, Jafford, & Jarrard, 1989) contrasting with deficits on a

delayed alternation task in a T-maze (Kessler & Markowitsch, 1981), and impairments on radial maze tasks (Kessler, Markowitsch, & Otto, 1982; Stokes & Best, 1990a, 1990b, 1990c).

1.6 Aims of the Present Study

In the light of the conflicting evidence from both human and animal studies, this series of experiments re-examined the effects of MD lesions in rats on the acquisition and performance of tests of working memory. In addition, the present study sought to compare the behavioural consequences of conventional lesions with neurotoxic lesions.

In this way the contribution, if any, from fibres of passage could be assessed. Two behavioural tasks were used. The first, delayed non-matching to sample, was chosen as this test of object recognition (Aggleton, 1985) appears closest in task demands to those tests of visual recognition shown to be sensitive to MD damage in monkeys (Aggleton & Mishkin, 1983b; Zola Morgan & Squire, 1985). These tests are in turn believed to be sensitive to diencephalic amnesia in humans (Aggleton, Nicol, Huston, & Fairbairn, 1988; Squire, Zola-Morgan, and Chen, 1988). The second, delayed forced alternation in a T-maze, was selected as

most of the conflicting results concerning MD lesions in rats refer to this or closely related tests of spatial working memory. By the use of these two tests of working memory, one spatial and the other non-spatial, I intended to examine further the possible contribution of MD to memory mechanisms in the rat.

CHAPTER TWO

TESTS OF NON-SPATIAL WORKING MEMORY

These experiments tested the ability of rats with lesions in MD to learn and perform tasks requiring non-spatial working memory. The rats were tested in a Y-maze (Fig. 3) in which the start-box and the two goal-boxes were removeable. On each trial the start-box matched one of the goal-boxes, but differed from the other. Selection of the unfamiliar box (nonmatching) was always rewarded. As both the start- and goal-boxes changed after each trial, this task taxed 'working memory' (Honig, 1978; Olton, Becker, & Handelmann, 1979).

In addition, a comparison was made between the behavioural effects of radio-frequency lesions, the conventional type, and those made by ibotenic acid, a neurotoxin which would spare fibres of passage within and around the target structure.

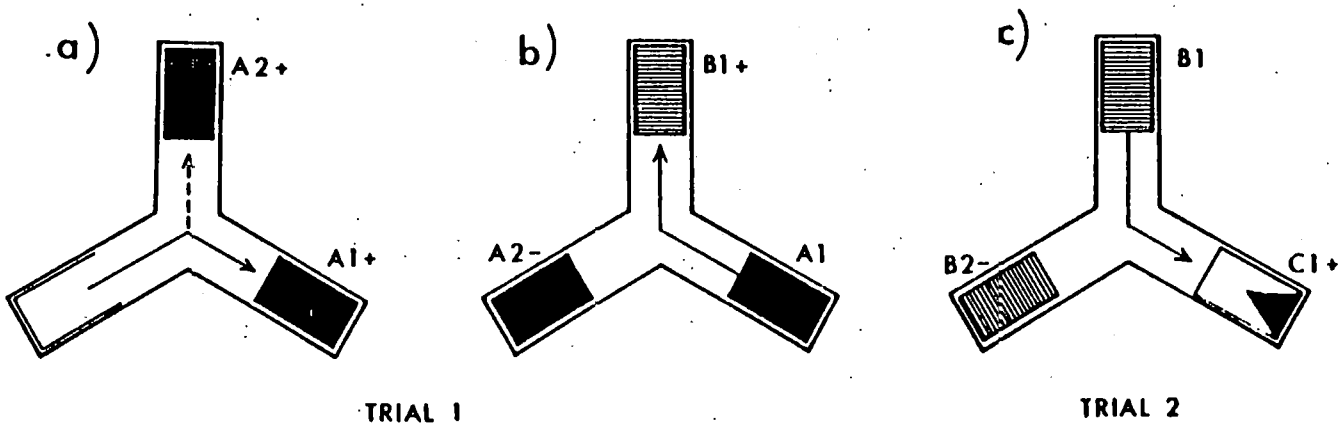

Fig. 3. Diagramatic representation of the Y-maze non-matching to sample procedure in which the animal was rewarded for choosing the novel goal box. Arrows indicate direction of correct responses (Aggleton, Hunt, & Rawlins, 1986).

2.1 General Methods

2.1.1 Subjects The subjects were 31 naive male rats of the pigmented DA strain (Bantin & Kingman, Hull), approximately three months old and 200 to 250g in weight at the beginning of the experiment. They were housed in individual cages in a room with a photoperiod of 14:10 hrs light/dark, and randomly allocated to one of three surgical groups. Following recovery from surgery and throughout the subsequent testing period they were maintained on approximately 15g of laboratory diet (Beekay Rat & Mouse, Bantin & Kingman, Hull) per day so that their bodyweights remained at no less than 85% of normal.

2.1.2 Surgical ProcedureAnimals in the group receiving chemical lesions were anaesthetised by a single intraperitoneal injection of 4ml/kg of Equithesin (containing 42mg/kg chloral hydrate and 9.7mg/kg pentobarbitone sodium), shaved, and placed in a stereotaxic headholder (David Kopf Instruments). The scalp was cut and reflected to expose the skull, part of which was then removed using a dental drill, thus exposing the dura above the sagittal sinus. Stereotaxic measurements were used to introduce the needle of a micro-syringe (Hamilton Instruments) into the thalamus of each hemisphere, and 0.6 microlitre of ibotenic acid solution (0.5 mg/100 microlitres, Sigma) dissolved in phosphate buffer (pH 7.2)

was injected into each mediodorsal nucleus. The stereotaxic coordinates relative to ear-bar zero, with the incisor-bar set at +5.0 relative to the horizontal zero plane, were A/P = +3.0mm, Ht.= +1.0mm, Lat.= +/-0.7mm. Each injection was made gradually over a 5 minute period per hemisphere, and the needle allowed to remain in situ for a further 5 minutes before being withdrawn. Sulphanilamide powder was applied and the skin sutured back into place.

Two-stage surgeries were carried out on four rats in this group in response to the toxicity of the ibotenic acid. There was a gap of between 7 and 13 days between the unilateral injections in the two hemispheres.

The animals receiving radiofrequency lesions underwent the same surgery as the ibotenic acid group, except that a steel electrode, insulated except for the tip (1.2mm), was substituted for the micro-syringe needle and a radiofrequency current (8 mA for 15 seconds) was passed in each hemisphere (Grass LM4 lesion maker).

Animals in the SHAM group underwent exactly the same procedure, with the exception that the needle or electrode was inserted to a point just short of the target nucleus (Ht = Ear-bar 0 +1.5mm) and withdrawn without any injection being made, or current passed. Post-operative management for all groups included heat and oxygen supplement, and injections of etamphylline (35mg/kg) and buprenorphine (0.15mg/kg).

2.1.3 Histological Procedure On completion of testing each rat was perfused intracardially with 5% formol saline. The brains were blocked, embedded in wax (Paraplast), and cut in 10u coronal sections. Every tenth section was mounted and stained with cresyl violet, a Nissl stain. Each adjacent section was reacted with luxol fast blue, a fibre stain. Each lesion was examined under light microscopy and the extent of cell loss plotted onto seven standard coronal sections (+6.2, +5.8, +5.2, +4.8, +4.6, +4.4, +4.0) from a stereotaxic atlas (Pellegrino & Cushman). A planimeter was used to calculate the total area of thalamic damage represented on these sections.

2.1.4 Apparatus A three-armed maze (Y-maze) was used, each arm of which was 13cm wide and 20cm high. Fifty pairs of hardboard boxes served both as start- and goal-boxes and fitted into the end of each arm of the maze, giving a total arm length of 26cm. The floors of the boxes, which extended towards the centre of the maze, began 8cm from a Y-shaped aluminium guillotine door at the centre. Food pellets (45mg Campden Instruments Ltd.) could be dispensed under the back of each box. Illumination was by a fluorescent room-light suspended 215cm above the apparatus. The luminant light level at the centre of the maze was 290 lux. The apparatus was identical in all respects to that used in previous

studies of recognition memory in rats. (Aggleton, 1985; Aggleton, Hunt, & Rawlins, 1985).

2.2 EXPERIMENT 1

NORMAL DELAYED NON-MATCHING TO SAMPLE

2.2.1 Method

Subjects - The 31 rats described in the section "General Methods" underwent Experiment 1, and were randomly allocated to one of three surgical groups: ibotenic acid lesions (MDibo, n = 10), radio-frequency lesions (MDrf, n = 8), and operated shams (SHAM, n = 13). Unfortunately, one of the animals in the SHAM group fell ill after the acquisition phase of the experiment, so its data were deleted from the results. This, therefore, gives an effective SHAM group size of 12.

Surgery and histology were carried out as described in the section " General Methods ".

Apparatus - was as described in "General Methods". The hardboard boxes which served as start/goal boxes had their floors and walls decorated in different colours and patterns, and the floors were lined with a variety of materials such as sandpaper, wood strips, wire mesh, metal sheet, and cloth. In addition, each pair contained identical

objects such as a plastic cup, a metal bracket, or a wooden block. In this way each pair was designed to be as similar as possible and yet as distinct as possible from any other pair (Fig. 4).

Behavioural Procedure - Following a period of pretraining, which involved handling the rats daily and training them to run in the Y-maze for food pellets, the experiment proper began. At the start of each session the rat was placed in an arm of the Y-maze with a blank hardboard box. The central door was raised and the animal allowed to choose between the two other arms which contained a matching pair of distinctive goal boxes (A1, A2; Fig. 3). The rat had made a choice when all four paws entered an arm, whereupon the guillotine door was lowered. On this first run the animal was rewarded with three 45mg food pellets (Campden Instruments) whichever box it entered. The animal was confined to this arm (A1) for a period of approximately 20s during which the other two test boxes were replaced. The central door was then raised revealing a familiar box (A2) in one arm and a novel box (B1) in the other. The rat was rewarded with three food pellets if it entered novel box B1, the rewards always being given after the animal had made its choice.

After 20s confinement in box B1 the second trial began. The central door was raised and the animal chose

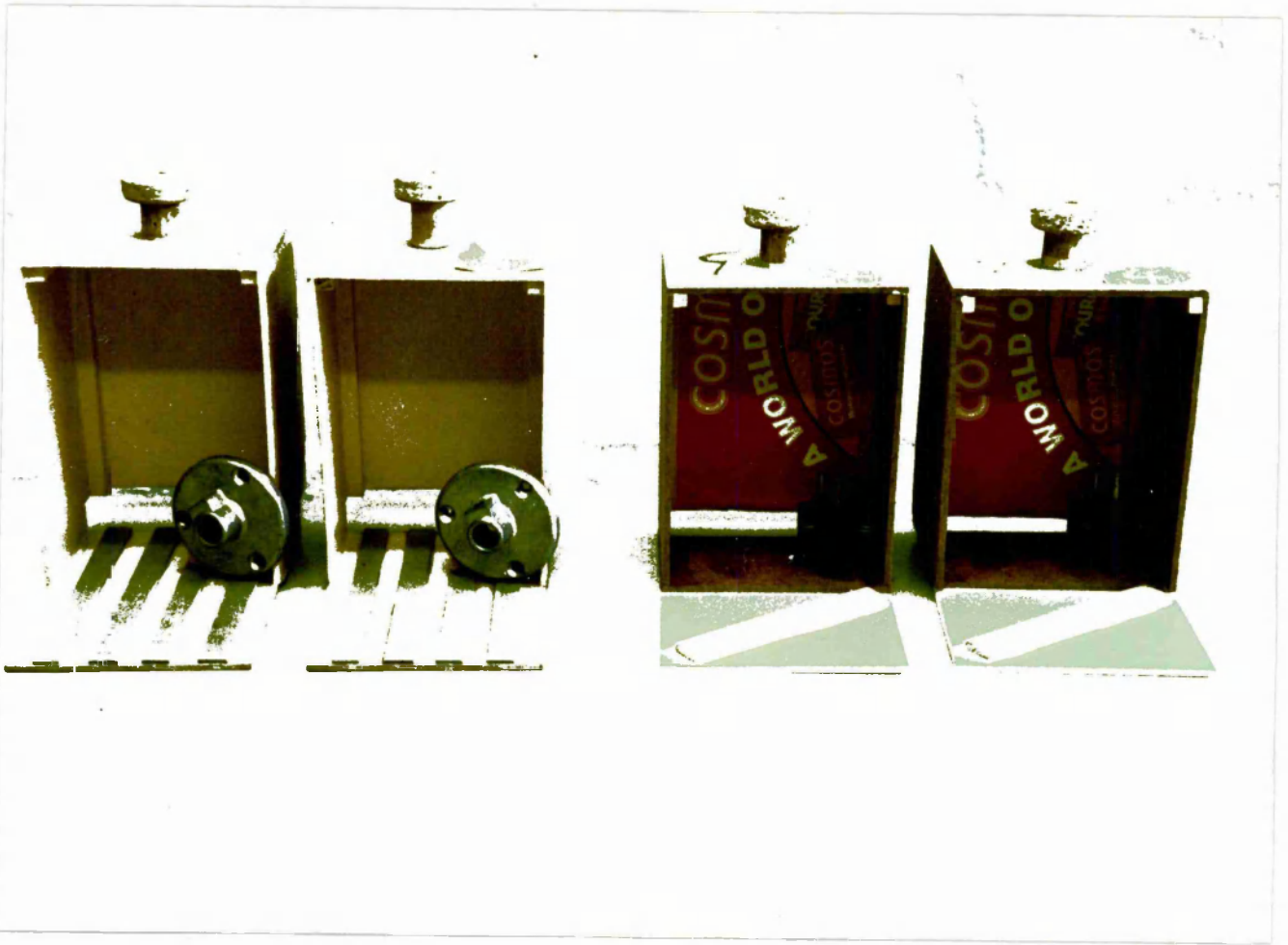

Fig. 4. Two pairs of start/goal boxes used in the Y-maze non-matching to sample experiments.

between the now familiar appearance of box B2 (negative) and a novel box C1 (positive). This procedure was repeated with pairs of new boxes for a total of ten trials during which the novel box was always rewarded. A balanced schedule determined whether the correct response was to the right or left. The sequence of test-boxes varied after every fifty trials so that on average any particular box would appear once every five sessions.

If an animal made an incorrect choice, correction trials were run with the same set of goal-boxes until the animal selected the novel box. During these correction trials the goal- boxes were rearranged so that entering the positive box required the same body-turn as in the test trial.

When a rat achieved a criterion score of 40 or more correct responses over five consecutive days (80%) it then moved on to the second phase of the experiment. If, however, a rat failed to reach the criterion score within 400 trials it was excluded from the remainder of Experiment 1. The second phase consisted of a further 150 trials in which retention intervals of '0's (as in acquisition), 20s, and 60s were imposed. For the two longer retention intervals the rats were again confined in the start-box for 20 seconds, but after this period had elapsed the start-box was removed and replaced by a blank box. The rat was not handled during this procedure but allowed to

step down from the start box onto the floor of the maze. The start-box was then immediately replaced by the blank box. Following a further 20s or 60s confinement in this box, the central door was raised, revealing the two goal-boxes; a novel one and one which resembled the start-box that had been removed prior to the delay. As before, the rat was rewarded for choosing the novel box, and an incorrect choice was followed by correction trials. This procedure required the rat to retain the memory of the start-box for at least 20 or 60 seconds. The rats received five days (50 trials) at each of the three conditions in a counterbalanced order.

2.2.2 Results

Histology - The MDrf group consistently showed extensive bilateral damage throughout the extent of the nucleus (Figs. 5 & 6), with the estimated extent of MD damage ranging from 38% to 86% (median MD damage = 74%). These lesions were centred in the medial subdivision of MD, although in nearly all cases there was considerable symmetrical damage to the central and lateral subdivisions of the nucleus as well. In all cases the lesion included the adjacent paraventricular nucleus. In two cases the lesion produced slight damage restricted to the most caudal portions of the anterior nuclei. In all animals the stria medullaris and the habenula were damaged

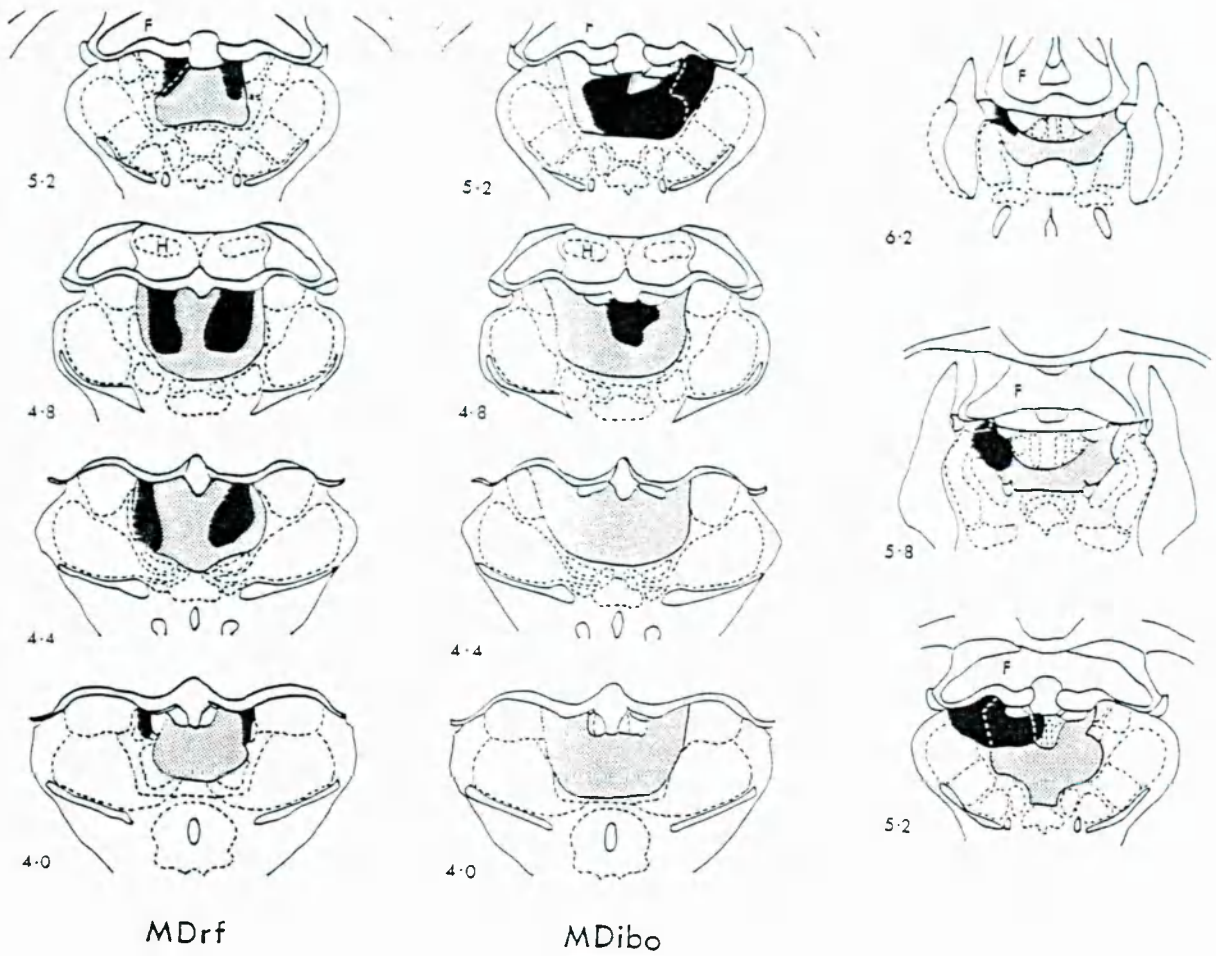

Fig. 5. Coronal sections depicting those cases with the largest and smallest lesions within the MDrf (left column) and MDibo (centre column) groups. The right column shows the extent of rostral thalamic damage in the subset of MDibo animals with anterior thalamic involvement. The numbers refer to the corresponding plates in Pellegrino and Cushman (1967). Abbreviations: F, fornix; H, hippocampus.

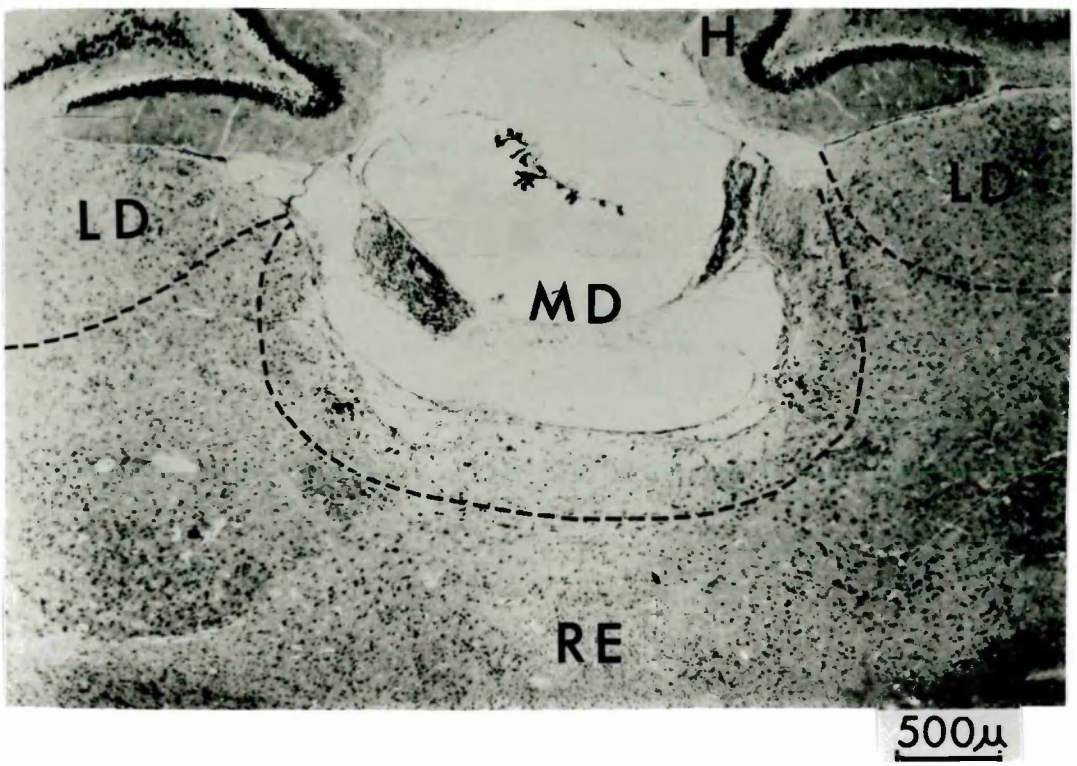

Fig. 6. Photomicrograph of coronal section (Nissl stain) showing the lesion location in the MDrf animal with the median extent of damage. Abbreviations: H, hippocampus; LD, lateral dorsal; MD, mediodorsal; RE, reuniens.

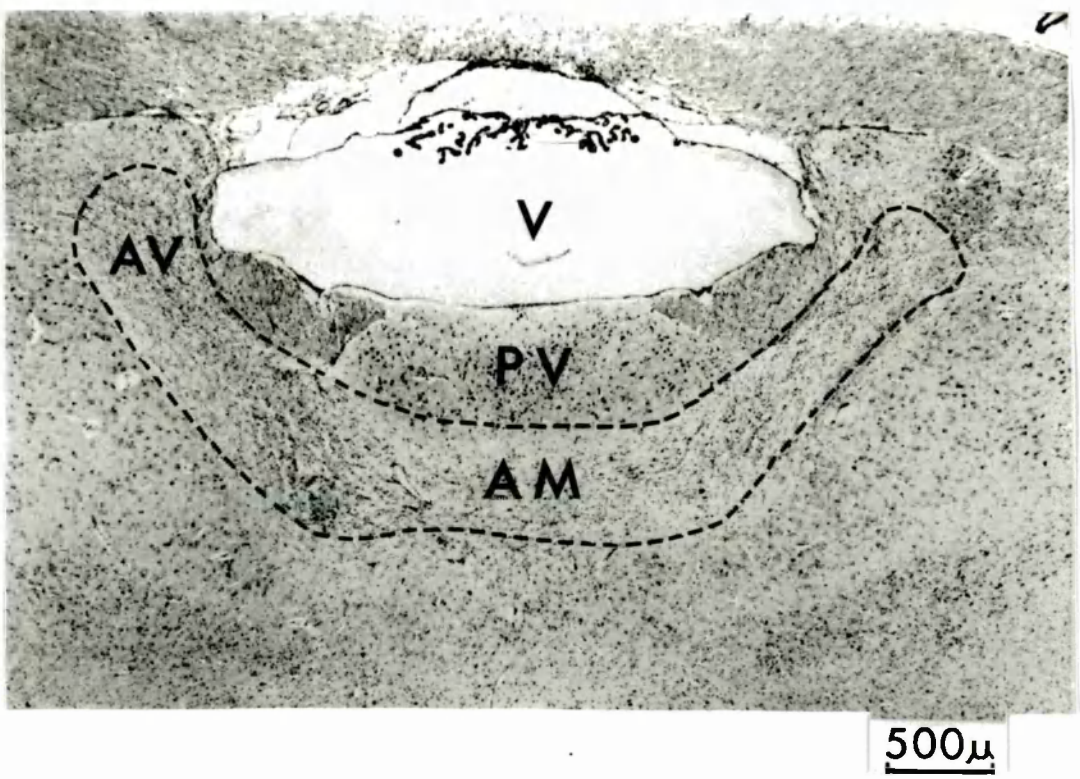

Fig. 7. Photomicrograph of coronal section (Nissl stain) showing the highly selective cell loss in the anterior thalamic nuclei which was present in some MDibo animals. Abbreviations: AM, anterior medial; AV, anterior ventral; PV, paraventricular; V, third ventricle.

bilaterally but in no case was there evidence of retrograde degeneration in either the mammillary bodies or the amygdala.

The lesions in the MDibo group were more variable and in general somewhat smaller than those in the MDrf group (Fig. 5). There was bilateral damage within the medial portion of MD in all cases and the percentage of total MD damage ranged from 12% to 98% (median MD damage = 34%). In all cases there was some involvement of the midline paraventricular nucleus although this was far less than that observed in the MDrf group. The stria medullaris appeared to be spared in all cases, although there was some partial involvement of the habenula in three animals. Additionally, in five of the ten MDibo cases there was substantial involvement of the anterior nuclei, which in two animals produced near-total bilateral cell-loss in the anterior medial (AM) and anterior dorsal (AD) nuclei, while the medial portion of the anterior ventral (AV) nucleus also showed marked cell-loss. In the remaining three animals the damage in the anterior nuclei was ipsilateral, often involving major portions of all three nuclei. In all five cases this rostral damage was restricted to the anterior thalamic nuclei, although it was accompanied by retrograde degeneration in the medial mammillary nucleus in the ipsilateral hemisphere. Examination of the sections which had been stained with luxol fast blue indicated that

the ibotenic acid had substantially reduced damage to fibres within MD.

Behavioural - While 11 of the 12 SHAM animals acquired the DNMS task within 400 trials, only half of the rats with MD lesions were able to reach the criterion score (MDrf, four out of eight; MDibo, five out of ten). These different distributions were found to be significant using the Fisher exact probability test (SHAM vs. MDrf $p<0.05$; SHAM vs. MDibo $p<0.05$). The severity of the acquisition deficit in the subgroup of nine MD animals that failed to acquire the task is reflected by the finding that their scores on the last 50 of 400 trials (mean = 30.4) did not differ from their scores on the first 50 trials (mean = 31.0). There was no evidence that the performance of those MDibo animals that received two-stage surgeries differed from the remainder of the group (two reached the DNMS criterion and two failed).

Comparisons between the total number of errors incurred in reaching the acquisition criterion, including those on correction trials, indicated that rats with thalamic damage made more errors than the SHAM animals (Kruskall-Wallis $H = 6.32$, $p<0.05$), nonparametric statistics being used in response to the arbitrary limit of 400 acquisition trials (Fig. 8). Further comparisons showed that both the MDrf ($U = 26$, $p<0.05$) and the MDibo ($U = 26$, $p<0.025$) groups made more errors than the SHAM group.

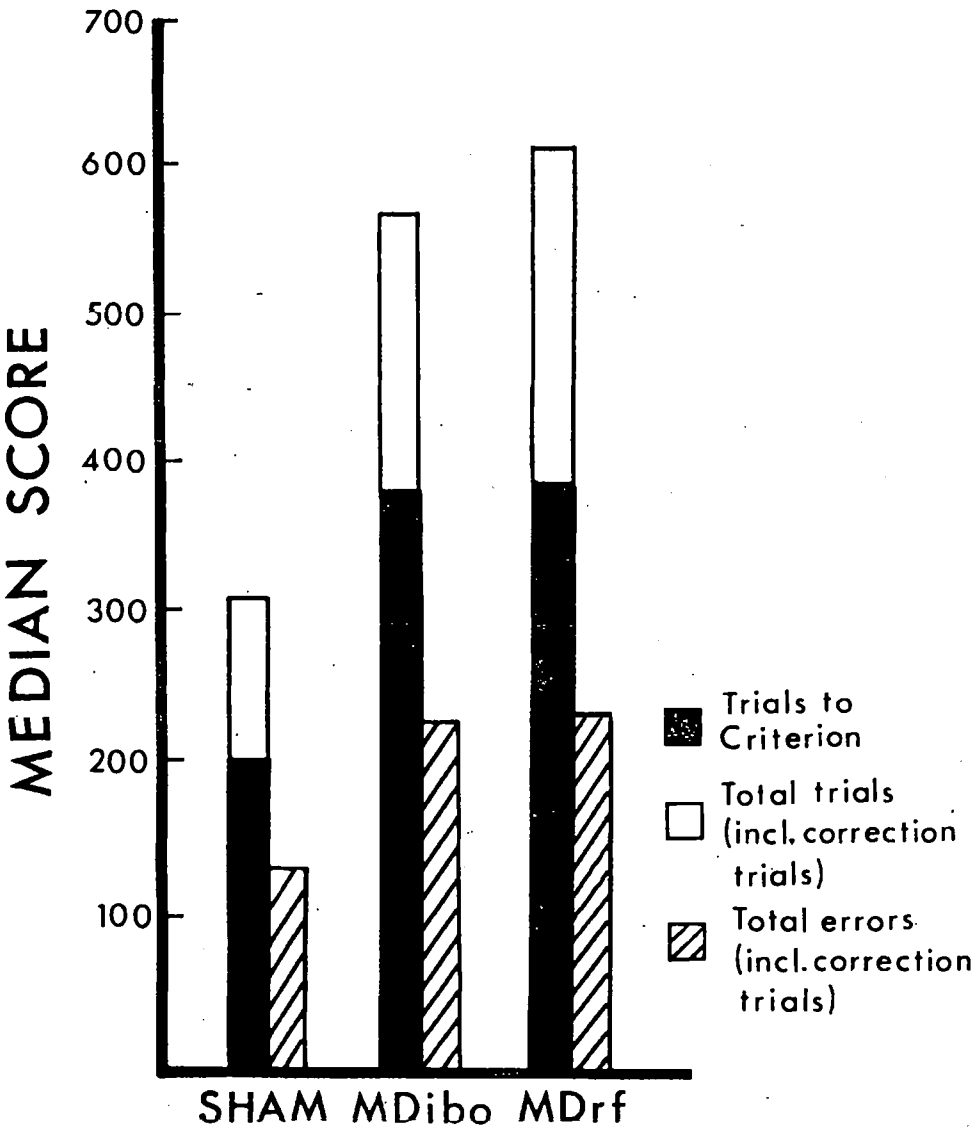

Fig. 8. Acquisition of the Y-maze task as measured by number of trials to criterion, total number of trials to criterion (including all correction trials), and total number of errors to criterion.

These group differences were evident after as few as 50 trials as the animals with thalamic lesions made fewer correct choices [$F(2,27) = 3.53$, $p<0.05$] reflecting the poor scores of both MD groups (mean scores; SHAM = 34.3, MDrf = 31.1, MDibo = 30.9). An examination of the number of correction trials per error made in the first 100 trials, a measure of body-turn perseveration, revealed no differences between the SHAM controls and those nine MD animals that acquired the DNMS task and those nine MD animals that failed ($F<1$).

It was found, however, that the subgroup of MDrf (n = 4) and MDibo (n = 5) animals that reached the acquisition criterion performed close to normal over the three retention delays; '0's (training condition), 20s, and 60s (Fig. 9). An analysis of variance confirmed that although increasing the retention interval did impair performance [$F(2,34) = 12.3$, $p<0.001$], there was no clear evidence of a lesion effect [$F(2,17) = 2.36$, $p>0.1$] or of an interaction [$F(4,34) = 1.41$]. When the standard '0's condition was removed evidence of a mild lesion effect over the two delay conditions was found although this did not reach significance [$F(2,17) = 3.55$, $0.1>p>0.05$]. No differences were found between the two MD groups. It should be noted that these analyses could only be applied to those animals that had reached the acquisition criterion and hence they only reflect a subset of animals

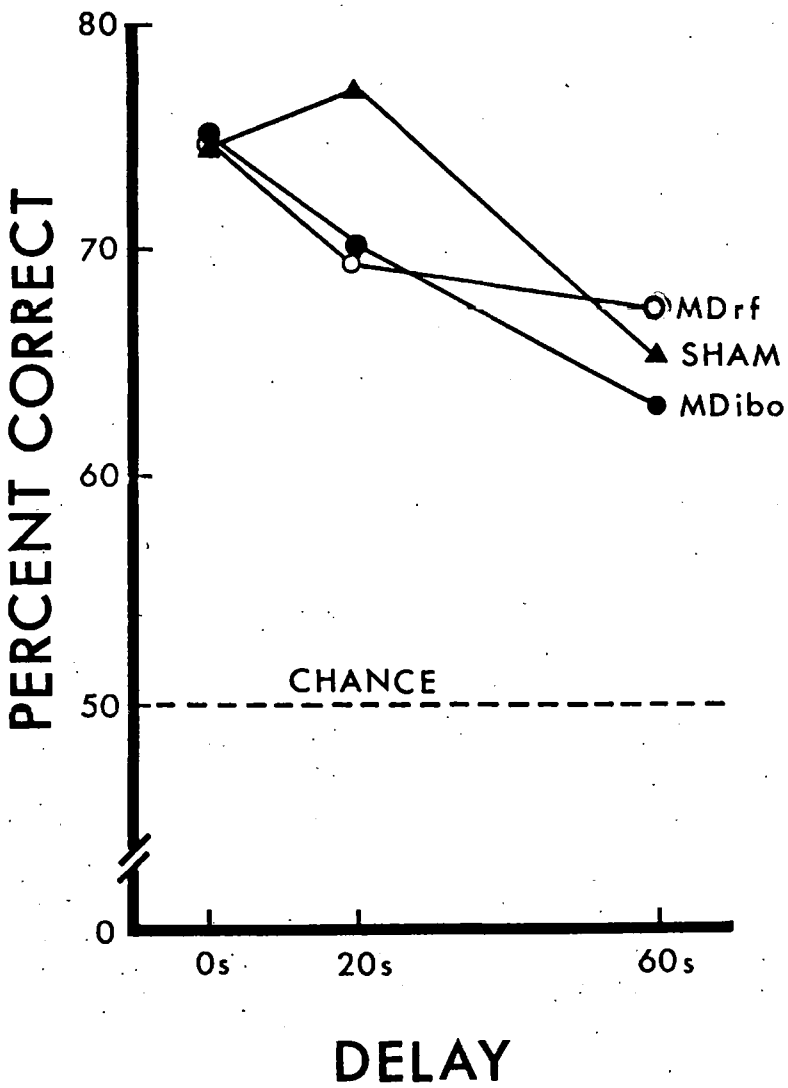

Fig. 9. Performance of the Y-maze task over increasing retention intervals between stimulus presentation and test of recognition.

from each MD group.

Subsequent histological examinations found no evidence that the failure of certain MDrf animals to master the DNMS task was linked with the presence of damage in any particular structure outside MD. As a consequence, the correlation was examined between extent of damage to MD measured from five standard coronal sections, and a measure of DNMS performance, which was the total number of correct trials in the first 100 trials (the highest number of acquisition trials that all MD animals received). This analysis revealed a significant, negative correlation (Spearman Rank $r = -0.72$, $p<0.05$) in the MDrf group, i.e. the greater the MD damage the poorer the DNMS scores.

There was greater variability in the extent and location of thalamic damage in the MDibo group. Nevertheless, there was once again a negative, but this time nonsignificant, correlation between extent of damage to MD and number of correct responses in the first 100 trials ($r = -0.47$, $n = 10$). There was, however, no evidence of a similar correlation with the extent of anterior thalamic nucleus damage and DNMS performance ($r = 0.1$).

The MDrf and MDibo groups were combined in a final analysis in which a significant, negative correlation was found between the extent of cellular MD damage and the number of correct trials in the first 100 trials ($r = -0.52$,

n = 18, $p<0.05$). Despite these correlations the apparently normal performance of those rats with MD lesions that did acquire the DNMS task was not simply a consequence of very small lesions as the extent of MD damage in these rats ranged from 13% to 82% of the nucleus.

2.2.3 Discussion

Animals in both groups with MD lesions were impaired on the acquisition of the DNMS task, although no differences were found between the MDrf and MDibo animals. The effects of the MD lesions upon acquisition were apparent using a number of different performance criteria, the deficit appearing as early as the first 50 trials. Furthermore, there was evidence that the extent of MD damage correlated with the size of the acquisition deficit. In contrast, those MDrf and MDibo animals that were able to master the task performed at close to normal levels over retention delays of up to 60s.

The emergence of the DNMS deficit after as few as 50 trials might at first suggest a sensory deficit, especially as the central division of MD is known to receive olfactory inputs (Cornwall & Phlillipson, 1988; Groenewegen, 1988). Although the use of pairs of boxes deliberately precluded the use of odour trails, the fact that the two goal-boxes presented to the rat on each trial always contained items made from different materials means that olfactory cues

could not be eliminated. While control tests have shown that rats do not rely on olfactory cues in order to perform the DNMS task (Aggleton et al., 1986; Huston & Aggleton, 1987), it is possible that such cues may add to the salience of the different pairs of boxes. Current evidence suggests that MD lesions do not affect detection thresholds in rats (Eichenbaum, Shedlack, & Eckmann, 1980; Slotnick & Kaneko, 1981), although impairments have been found with olfactory discriminations (Eichenbaum et al., 1980; Slotnick & Kaneko, 1981; Staubli, Schottler, & Nejat-Bina, 1987). These findings have been interpreted as indicating a procedural learning deficit rather than the consequence of a specific olfactory detection deficit (Staubli et al., 1987). However, a control experiment was run in order to explore this possibility further, and is described in the Appendix.

Consistent with the notion of a procedural learning deficit was the fact that the MD rats could discriminate between the stimuli and yet were often unable to master the nonmatching rule. There was, for example, no difference between the performance of the SHAM animals on the very first 20 DNMS trials and that of the nine animals with MD lesions that eventually failed to acquire the task ($t<1$), even though the scores of both groups were significantly above chance (minimum $t = 3.10$; $p<0.01$). This comparison is of interest because normal rats show a spontaneous

preference for novel boxes which is consistently detectable after only 20 trials (Aggleton, 1985; Aggleton et al., 1986), and which must reflect an ability to distinguish between the test stimuli. Similarly the same nine MD animals performed significantly above chance on the first 50 trials even though they were unable to improve their scores over 400 trials. Furthermore, there was no evidence that those MD animals that failed to acquire the DNMS task showed a tendency to perseverate their body-turns. Taken together these findings suggest a learning deficit.

The lack of any difference between the MDrf and MDibo groups on the DNMS task strongly indicates that damage to cells and not to fibres of passage within MD is responsible for the acquisition deficit. This conclusion is supported by evidence of significant correlations between the extent of MD damage and performance on the recognition task. Such correlations were found for both the MDrf group and for the combined MDrf and MDibo groups. The location of the lesions in the MDibo group provides some evidence that damage to the medial portion of the nucleus, which is connected with a large range of limbic structures and related cortical regions such as the prelimbic, infralimbic, cingulate, orbital, and insular cortices (Cornwall & Phillipson, 1988; Groenewegen, 1988; Krettek & Price, 1977), was particularly important in producing the DNMS deficit. Confirmation of this will,

however, have to await comparisons with the effects of lesions restricted to either the lateral or medial sections of MD.

2.3 EXPERIMENT 2

DELAYED NON-MATCHING TO SAMPLE - REPEAT BOXES

One feature of the object recognition test (Experiment 1) is that it minimized intertrial (proactive) interference by the use of multiple start/goal boxes which were changed after each trial, and only repeated after every fifth day. As a consequence, it is unlikely that the animals were confused by previous trials. In order to increase the difficulty of the memory component of the task, and to establish whether proactive interference might reveal a difference between the experimental groups, the intrasession interference was increased. This was done by repeating the recognition task using a restricted set of start/goal boxes.

2.3.1 Method

Subjects - The subjects were all rats which had reached criterion and subsequently completed Experiment 1 (MDibo n = 3, MDrf n = 4, SHAM n = 7).

Surgery, histology, and apparatus were as described in 'General Methods' and Experiment 1.

Behavioural Procedure - Testing consisted of ten daily sessions in the Y-maze, each session comprising twelve trials using the same start/goal boxes and general testing method as Experiment 1. Half of these sessions were normal with session-unique stimuli being presented on each trial, but on alternate sessions the rats were tested with a limited set of just four pairs of goal boxes, each set of four being different for each of the five sessions. During these sessions each pair was used as the correct "unfamiliar" stimulus three times. On their first appearance (trials 1-4) the four goal-boxes were truly unfamiliar, but on the remaining eight trials the boxes reappeared twice in a pseudo-random order so that the number of intervening trials varied between one and five. As a consequence, on trials 5-12 the rat was required to make a judgement of relative recency rather than recognition.

In all other respects the testing-conditions, including correction trials, were precisely the same as in Experiment 1.

2.3.2 Results

Figure 10 shows the performance of the animals over 4 normal sessions and 4 sessions using repeated stimuli. Only the last 8 trials of each session were considered, as it is only on these trials that repetition of the test

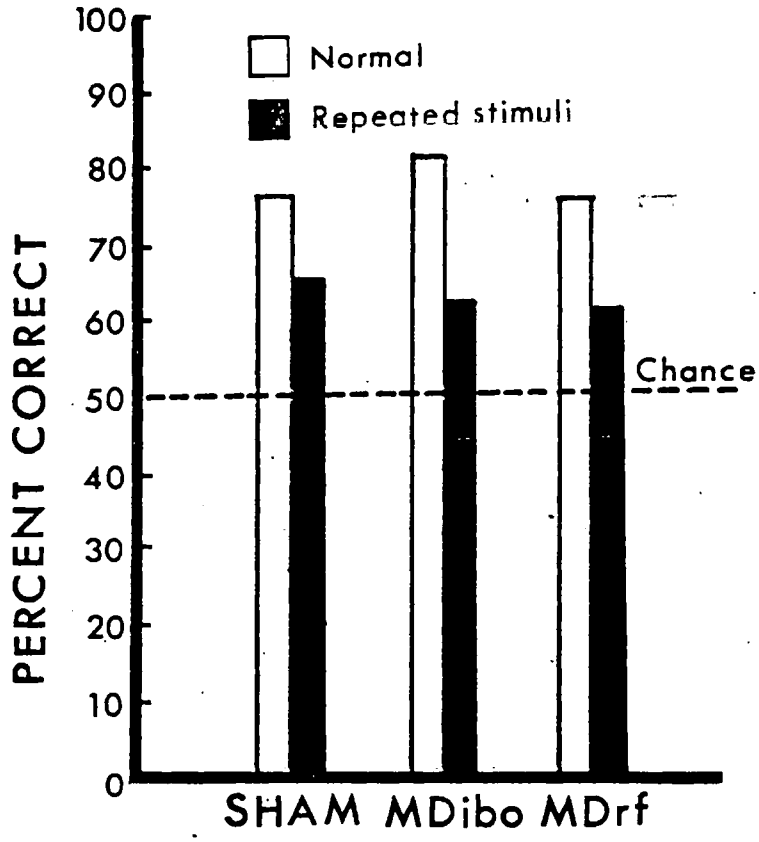

Fig. 10. Performance of the Y-maze task on days using repeated stimuli, and on days using the normal presentation of stimuli.

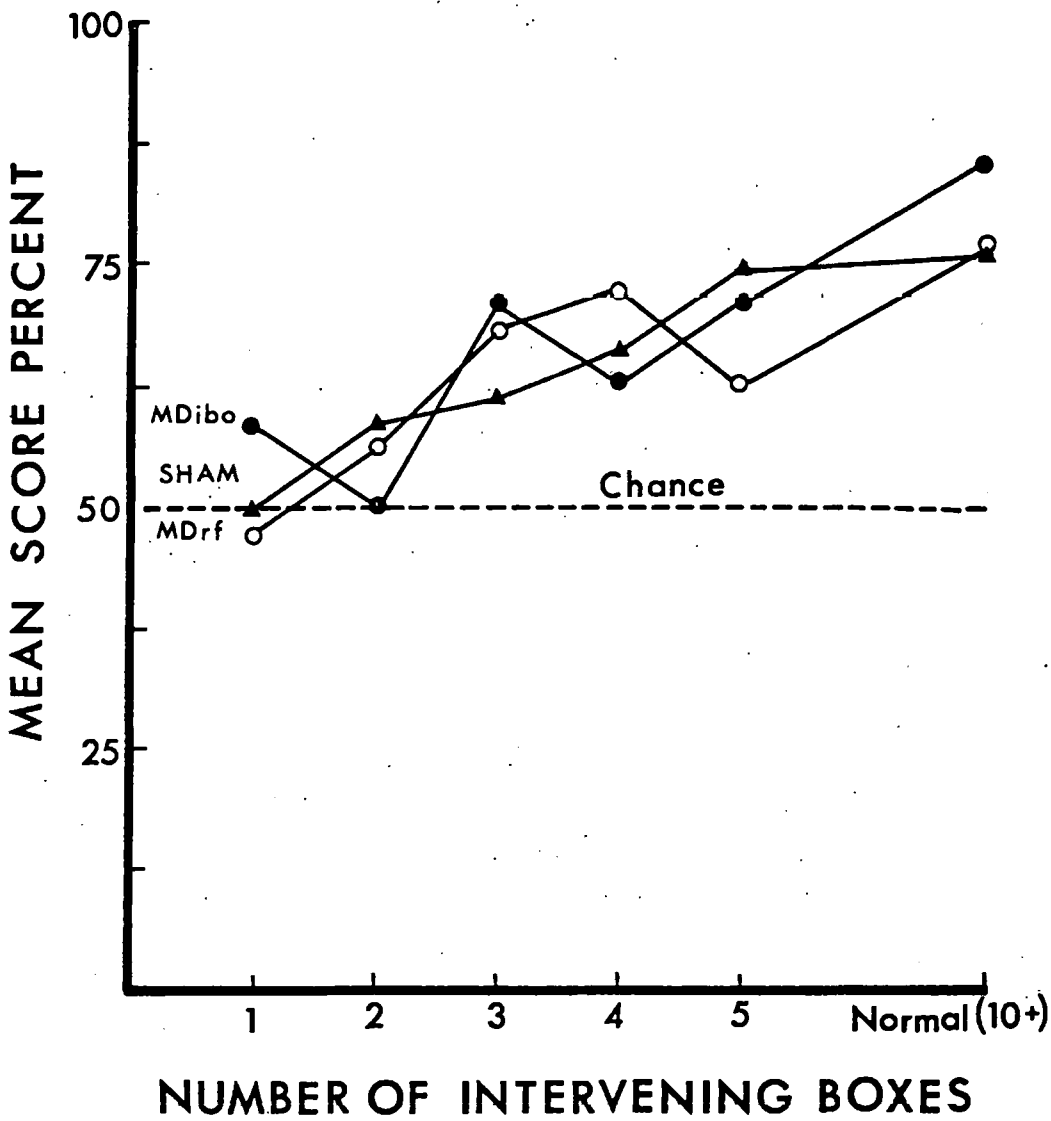

Fig. 11. Performance of all three groups of the Y-maze task using repeated stimuli.

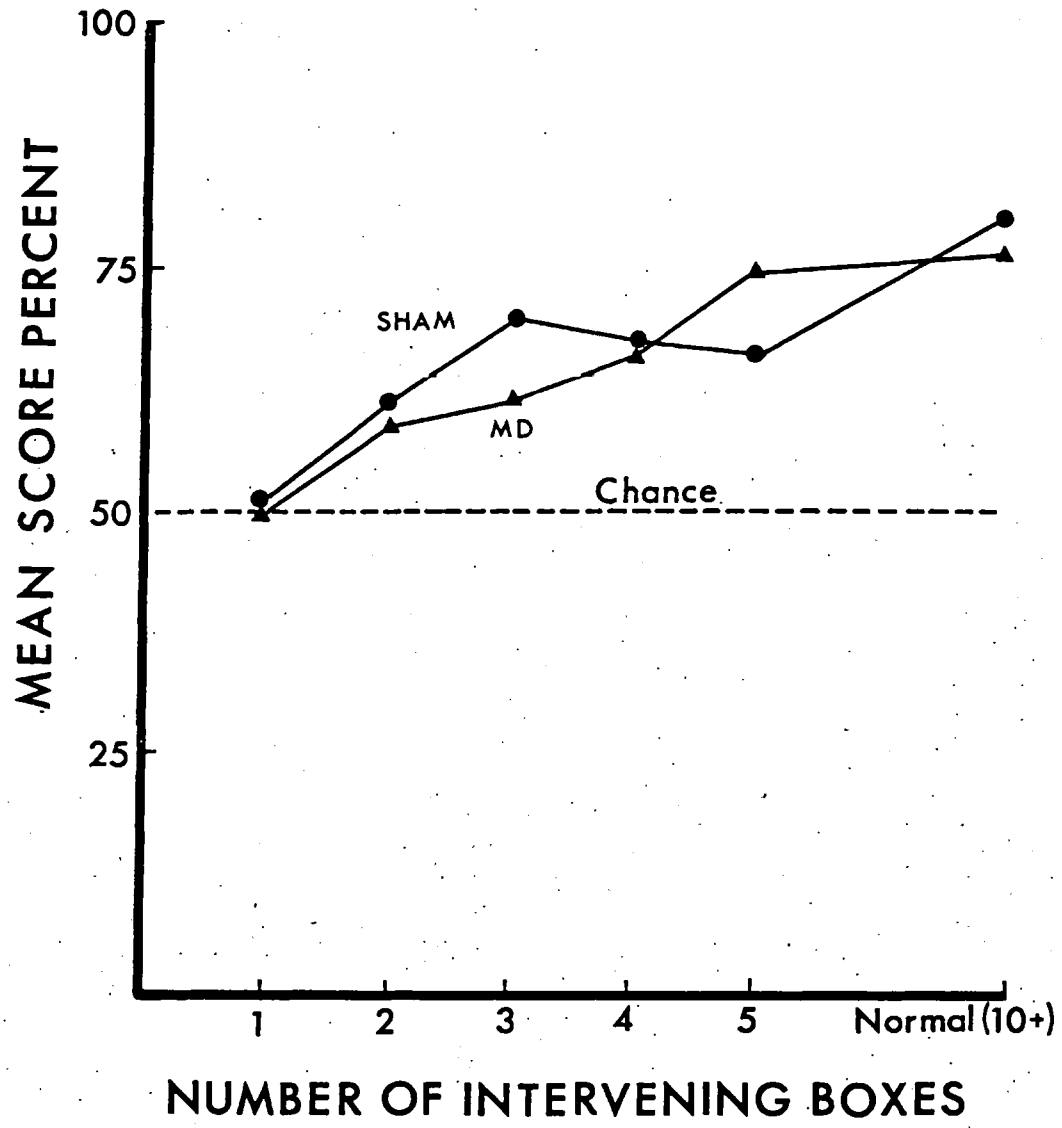

Fig. 12. Performance of the Y-maze task using repeated stimuli; MD groups combined.

stimuli occurred. An analysis of variance showed that on comparing the scores for the two different kinds of session there is no effect of lesion [$F(1,5) = 1.31$, $P = < 0.25$], but that there is a significantly reduced performance on the sessions using repeat stimuli ($F(1,5) = 41.4$, $P < 0.01$]. Given this lack of difference between the lesion groups, their results were then combined and compared against the SHAM group in the same way. This showed again that the reduction in performance on repeat days was significant ($F(1,2) = 7.5$, $P < 0.025$], and that there was no difference between the combined MD group and the SHAM group [$F < 1$]

Figure 11 shows the decline in performance by all 3 groups as the number of intervening boxes decreases and the degree of proactive interference increases. An analysis of variance again shows the reliability of the decline in performance as highly significant on both MDibo versus MDrf and combined MD groups versus SHAM [$F(4,20) = 1.82$, $P < 0.1$, and $F < 1$], and no difference in performance between the two lesion groups [$F < 1$], or between combined lesion groups and SHAM group [$F < 1$].

2.3.3 Discussion

This experiment tested whether the animals were sensitive to proactive interference, and whether either or both of the lesion groups were more affected by this increase in difficulty of the task than the SHAM animals.

The results show that the animals all showed a marked decrease in performance in the presence of proactive interference, but that this does reveal any working memory deficit in animals with MD lesions.

CHAPTER THREE

TESTS OF SPATIAL WORKING MEMORY

These experiments tested the ability of rats with lesions in MD to learn and perform a task requiring spatial working memory. The rats were tested in a T-maze in which they performed forced alternation tasks with delays for food reward, and in which the trials were organised in both spaced and massed regimes.

3.1 EXPERIMENT 3

DELAYED FORCED ALTERNATION - SPACED TRIALS

3.1.1 Method

Subjects - The subjects were the same as those that underwent the experiments in Chapter 1, except for one MDibo animal which fell ill at the start of this experiment and was therefore excluded. The experimental groups therefore comprised MDibo (n = 9), MDrf (n = 8), SHAM (n = 12).

Apparatus - The T-maze (Fig. 13) used in this task had an aluminium floor 10cm wide and clear acrylic walls 17cm high. The stem of the maze was 80cm long with an aluminium guillotine door 33cm from the beginning. The cross-piece was 136cm long with a food well 4 cm in diameter and 0.75cm deep in the floor at each end. The maze was supported on stands 93cm high and was illuminated by fluorescent room-lights suspended 92cm above the apparatus. At the choice point and food wells the luminant levels were 320 and 280 lux respectively. Testing was carried out in a different room from that used in Experiment 1, and in it tables, cage racks, sinks, and a computer provided salient spatial cues.

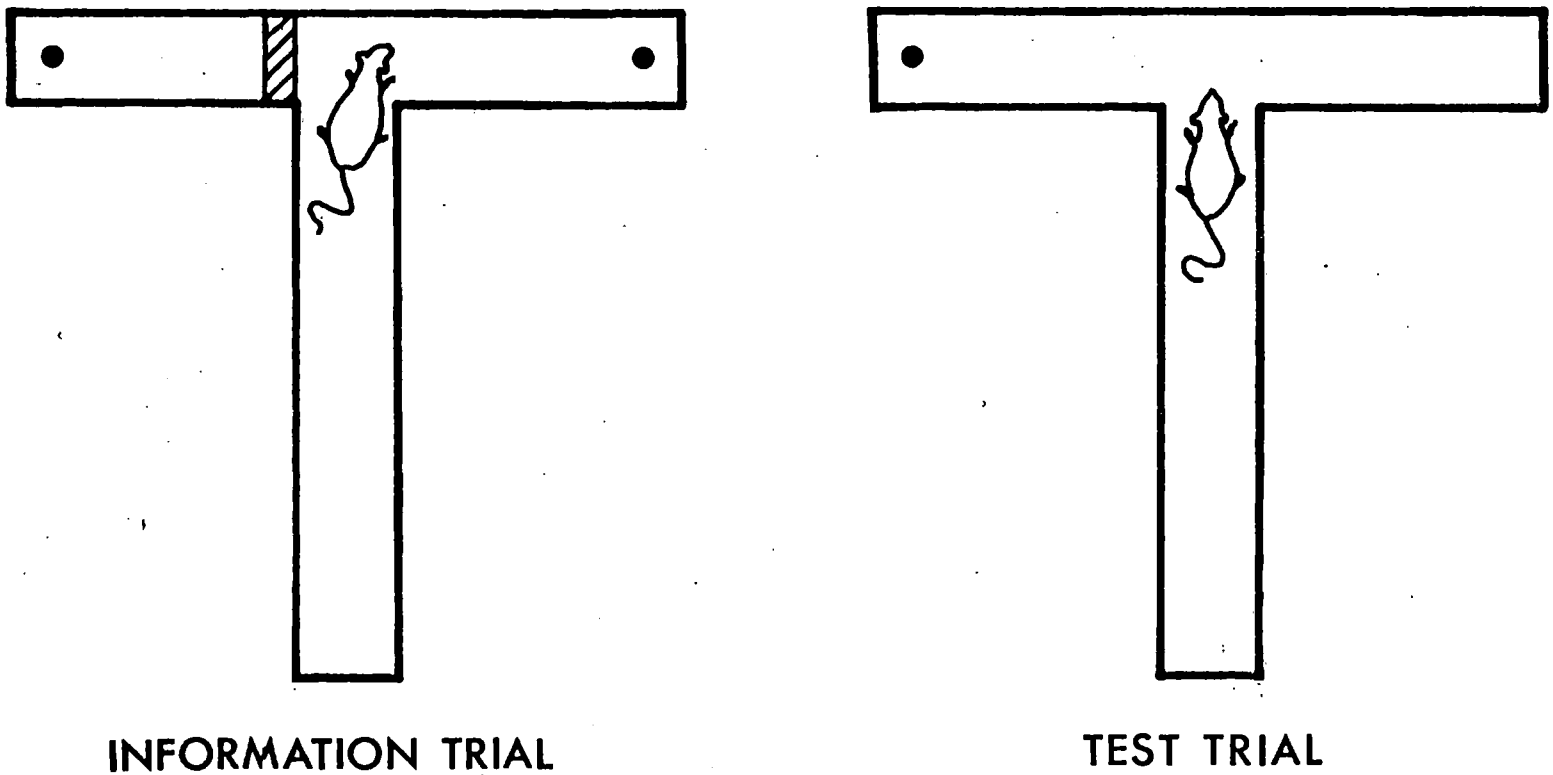

Fig. 13. Diagramatic representation of the T-maze spatial forced alternation task in which the animal was rewarded for alternating after a forced-choice information run.

Behavioural Procedure - Testing began on average 30 days after completion of Experiment 1. Each rat required only 1 or 2 days of pretraining before it would reliably run down the stem of the maze to find food pellets in both the food-wells.

Each trial in this experiment consisted of two stages; an 'information' run and a 'test' run. To begin each trial, three food-reward pellets (45 mg) were placed in each food well and a wooden block was used to close off one arm of the maze adjacent to the choice-point. The rat was then placed at the start-point and the guillotine door was raised (Fig. 13). On this 'information' run the animal was forced by the wooden block to enter the open arm of the T-maze and was allowed to eat all of the pellets in the food well. No retracing was permitted. The rat was then picked up and placed back in the start-box, the wooden block removed, and the door raised so that the rat was able to make a second run. The delay between the two runs was approximately ten seconds. On this second or 'test' run the rat was free to enter either arm, and was deemed to have chosen when it had placed both hind feet in either of the goal arms, whereupon the wooden block was put behind it to prevent retracing. If the rat had alternated, i.e. entered the other arm to the one it had chosen on the information run, it was allowed to eat the food and was then returned to its cage. If it had chosen the same arm, i.e. had not

alternated, the rat was confined to the arm for approximately ten seconds before being returned to its cage.

The rats were tested in groups of three or four, with each rat having one trial in turn. This 'spaced' method meant that there was an inter-trial interval of three to five minutes. Each rat received six trials per day, each consisting of two runs through the maze. The six trials consisted of three left and three right forced turns in a mixed order, and consecutive rats in a group were run on different schedules of turns. Testing was carried out for a total of six days (36 trials).

These six sessions were immediately followed by the second phase of this experiment in which three different retention delays (10s, 30s, and 60s) were imposed between the information and test runs, again using the spaced schedule. The rats were returned to their cages for the duration of these delays. As before the rats received six trials per session and were tested in groups of three or four so that the intertrial interval was between three and five minutes. The three delay conditions were mixed equally within each session, and the experiment comprised ten such daily sessions.

3.1.2 Results

All rats were able to acquire this task relatively quickly, but the MDibo group made more errors in acquisition

than the MDrf or SHAM groups (Fig. 14). However, this difference when analysed in a session-by-session comparison failed to reach significance [$F(2,26) = 3.24$, $0.1>p>0,05$]. The same analysis also indicated that there was no clear session effect [$F(4,104) = 2.14$], and no lesion x session interaction [$F(8,104) = 1.79$].

During the subsequent 20 trials at each of three delay conditions in the second phase of the experiment, performance declined, as expected, in response to the lengthening intervals [$F(2,52) = 34.9$, $p<0.001$]. However, there was no evidence of a lesion effect [$F<1$], or of a lesion x delay interaction [$F(4,52) = 1.19$].

The MDibo group could be divided into those with or without marked cell loss in the anterior thalamic nuclei, and inspection of the data showed a very clear correlation between anterior nucleus damage and performance. The two animals with the greatest bilateral cell loss in AM, AD, and parts of AV, which was combined with retrograde degeneration in the medial mammillary bodies, made the fewest correct responses under delay conditions of all the 28 animals in the study (68% and 67%, out of 60 trials). In addition, one of the two animals with extensive unilateral anterior nucleus damage, involving all of AD, AM, and AV, achieved the next lowest total (84%). Although the fourth animal, with unilateral damage restricted to just AV and AD, achieved a normal set of scores (89%), the overall scores

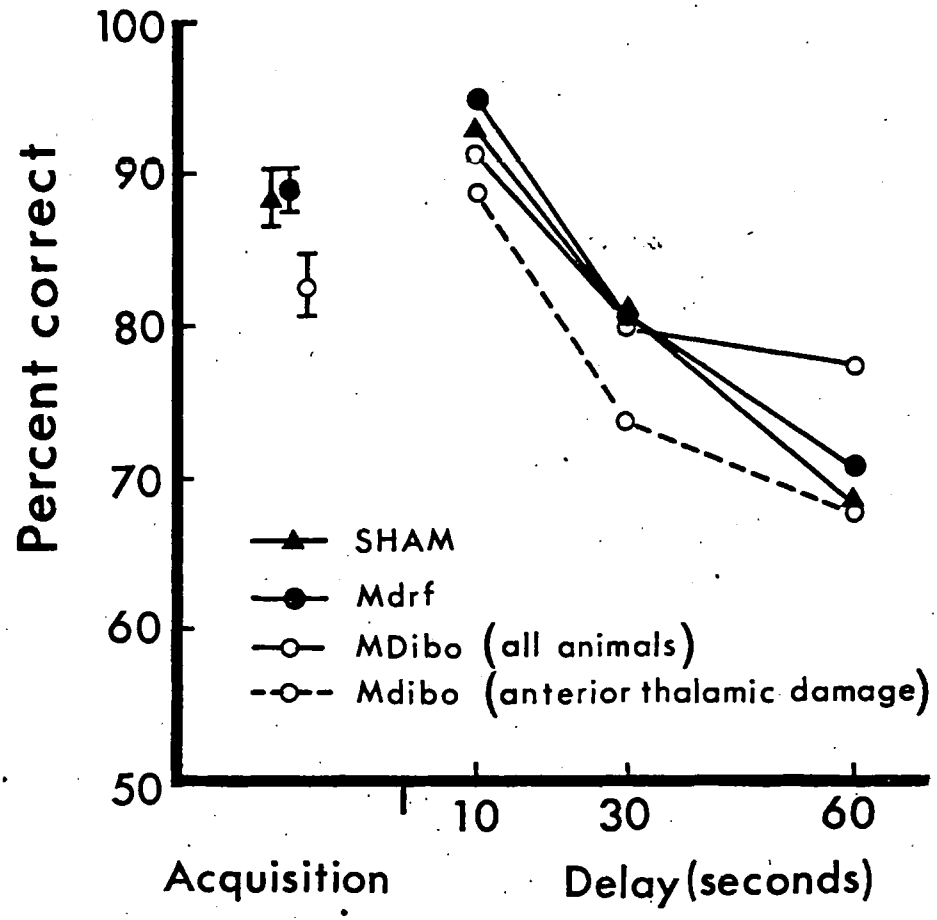

Fig. 14. Spatial forced alternation: Acquisition (mean performance over five test sessions) and performance with increasing retention intervals using spaced trials. Vertical bars show standard errors.

of this subgroup of four MDibo rats were significantly lower than those of the remaining five MDibo animals (Mann-Whitney, $U = 2$, $p = 0.032$). The MDibo animals with anterior thalamic damage were not differentially affected with increasing delays, but performed at a lower level on all of the delays (Fig. 14).

There was no evidence that the MDibo animals without anterior nucleus damage were impaired on these T-maze tasks. This is consistent with the finding that within the MDibo group the correlation between total MD damage and total errors on the delay conditions was negative ($r = -0.53$) while the correlation with the extent of damage to the anterior nuclei was positive ($r = 0.73$, $n = 9$, $p<0.025$).

3.1.2 Discussion

No evidence was found that MD damage disrupted performance on this task using delays and spaced trials. Deficits were, however, found in those MDibo rats with appreciable damage to the anterior thalamic nuclei, the deficit being apparent at all conditions.

The high level of performance of animals with extensive MD damage prompted a re-examination of the link between this nucleus and impairments in tests of spatial working memory. In particular, the present findings suggest that impairments reported after MD damage may, in some cases, reflect damage extending rostrally into the

anterior thalamic nuclei or ventrally into the mammillothalamic tract (Sutherland & Rodriguez, 1989; Thomas & Gash, 1985). This would, in turn, be consistent with the fact that there are dense hippocampal projections to the anterior thalamic nuclei and mammillary bodies.

Of ten other studies of spatial working memory using either a T-maze or an eight-arm radial maze four reported normal levels of performance (Green & Naranjo, 1986; Kolb et al., 1982; Olton, 1978; Tigner, 1974) while six reported deficits (Brito et al., 1982; Kessler et al., 1982; Means, Harrell, Mayo, & Alexander, 1974; Stokes & Best, 1988; Vicedomini et al., 1982; Weiss & Means, 1980) following MD lesions. In half of these six studies it is evident that in nearly all animals the lesions involved one or more of the anterior thalamic nuclei (Kessler et al., 1982; Stokes & Best, 1988; Weiss & Means, 1980).

In one of the remaining three studies the extent of the spontaneous alternation deficit correlated closely with the extent of habenula damage but not with damage to MD (Means et al., 1974). Furthermore, many of the animals suffered some bilateral damage to the dorsal hippocampus. In a second study (Vicedomini et al., 1982) the histological reconstructions strongly indicate that many cases received some damage to the anterior thalamic nuclei, and that in some cases the lesions extended ventrally into the mammillothalamic tract. In the third study (Brito et

al., 1982) it was stated that the anterior thalamic nuclei were spared, although the mammillothalamic tract was transected in two of the seven rats. Unfortunately, only a single micrograph at a single level was provided to illustrate the MD lesions (Brito et al., 1982).

These results seem to imply that lesions restricted to MD do not, by themselves, impair performance on tests of spatial working memory. However, the three studies (Brito et al., 1982; Vicedomini et al., 1982; Weiss & Means 1980) that found impairments using a T-maze had also used massed rather than spaced trials. It was therefore decided to run this test using massed trials in order to increase the difficulty of the task.

3.2 EXPERIMENT 4

DELAYED FORCED ALTERNATION - MASSED TRIALS

This experiment was run immediately after and as a response to Experiment 3. The aim of using massed trials as well as delays was both to increase the difficulty of the working memory component of the task, thus exposing any more subtle impairments that might exist in the MD lesion groups, and also to be as consistent as possible with previous studies (Brito et al, 1982; Vicedomini et al, 1982; Weiss & Means, 1980) which used this method and reported impairments in MD rats.

3.1.1 Method

The method used for this experiment was exactly the same as that used in Experiment 3, but with the following changes made to the behavioural procedure.

Each rat was given six trials per day in a massed rather than a spaced regime, i.e. each rat had six consecutive trials with only a 30 second intertrial interval between a test run and the next information run. Retention delays of either 10 seconds or 30 seconds were placed between the information and test runs. The rats

received ten sessions in which these two different delays were mixed equally within each session.

3.2.2 Results

As in Experiment 3, there was a massive effect with delay (Fig. 15) [$F(2,26) = 81.75$, $p<.001$], but no clear lesion effect [$F(2,26) = 1.33$]. There was, however, a significant lesion x delay interaction [$F(2,26) = 6.94$, $p<.01$] which reflected the steeper decrement in performance in the MDrf animals (Fig. 15). This was confirmed by a pairwise comparison between the SHAM and MDrf groups which revealed both a lesion effect [$F(1,18) = 4.89$, $p<.05$] and a significant interaction [$F(1,18) = 7.04$, $p,.025$].

The subgroup of MDibo animals with anterior thalamic damage again showed clear impairment in a very similar pattern to Experiment 3, with the two poorest performers (57% and 63%) being those two with the largest anterior bilateral cell loss. The animal with extensive but unilateral anterior damage again performed poorly, achieving the fourth lowest score (78%), and the animal with the smaller unilateral anterior lesion again performed normally (90%).

Finally, a comparison was made of the performance of all MD animals on spatial tasks against non-spatial performance. Total errors on all T-maze conditions were compared with total errors to acquisition on the Y-maze

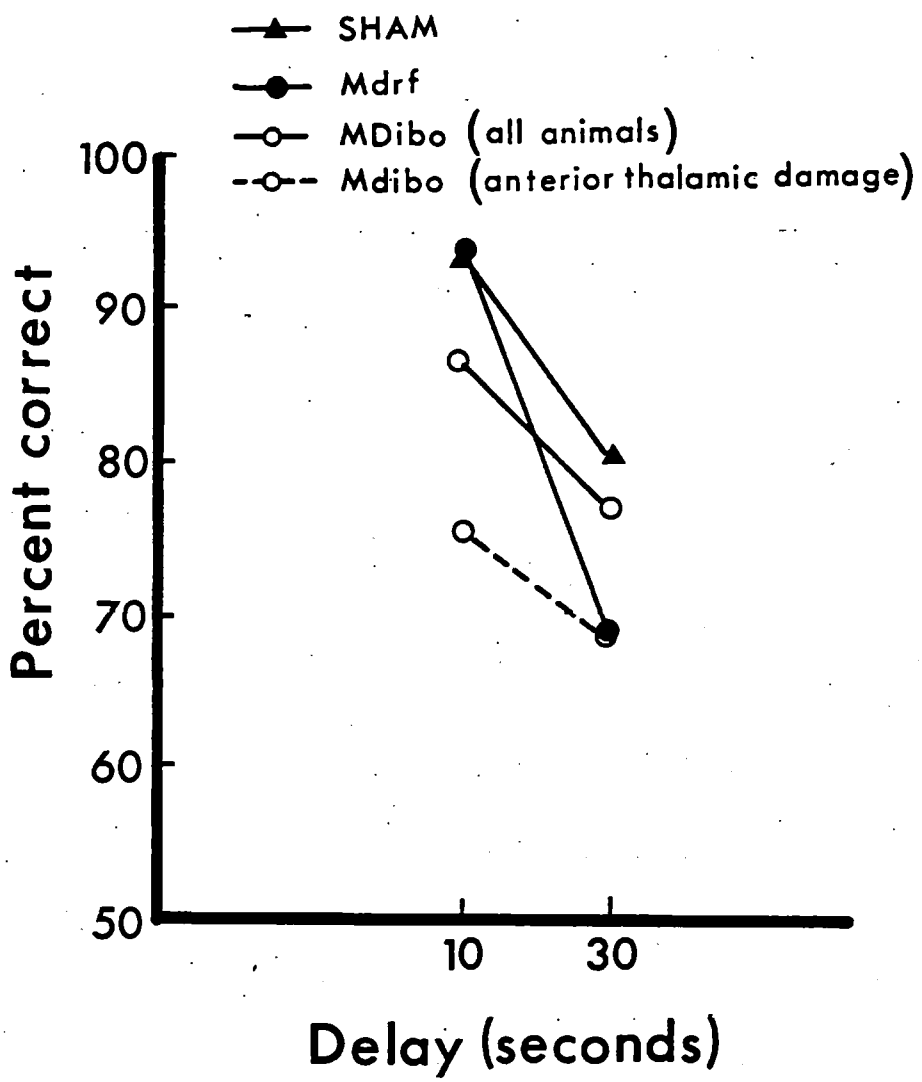

Fig. 15. Spatial forced alternation: Performance with increasing retention intervals using massed trials.

task, and no evidence of correlation was found [r(17) = 0.13].

3.2.3 Discussion

The results of this experiment seem to imply that a combination of delay conditions and massed trials can indeed result in rats with MD lesions being impaired on a test of spatial working memory, the MDrf animals showing a faster rate of forgetting than the other groups (Fig. 15). However, the fact that the MDibo group remains unimpaired under these conditions again questions whether the deficits reported by previous studies (Brito et al., 1982; Kessler et al 1982; Means et al, 1974; Stokes & Best, 1988; Vicedomini et al, 1982; Weiss & Means, 1980) can confidently be attributed to damage to cells within MD.

The subgroup of animals with anterior nucleus damage in the present study was again clearly impaired (Fig. 15), thus reinforcing the findings in Experiment 3, and the subsequent suggestion that the deficits found in three of the six studies above (Kessler et al., 1982; Stokes & Best, 1988; Weiss & Means, 1980) may be attributable to the anterior damage they reported. In the three remaining studies (Brito et al, 1982; Means et al, 1974; Vicedomini et al, 1982) the lesions were of the non-neurotoxin type and, as reported in Experiment 3, all had included damage to fibres in some of the lesion group animals.

In conclusion, the case that lesions restricted to MD can impair tests of spatial working memory can only be described as unproven. In the present study the only condition that produced an impairment was the combination of massed trials and increased retention delays; no deficit being observed when these two factors were examined separately. The present findings, combined with the evidence that damage to the anterior thalamic nuclei or the mammillothalamic tract (Sutherland & Rodriguez, 1989; Thomas & Gash, 1985) can disrupt similar spatial tasks, points to the conclusion that MD damage may not, on its own, be sufficient to impair spatial working memory and that some deficits reflect inadvertent damage to adjacent regions or possibly to fibres of passage.

CHAPTER FOUR

DISCUSSION

The primary aim of this study was to examine the effects of lesions in the nucleus medialis dorsalis on two types of working memory tests, object recognition and spatial alternation. Both used a delayed non-matching to sample rule, although the former was non-spatial and the latter spatial. The secondary aim was to determine whether damage to fibres of passage contributed to the effects of MD damage.

It was found that MD lesions, produced by either radiofrequency or neurotoxin, significantly impaired acquisition of the nonspatial DNMS task. However, those animals that were able to master the task could subsequently maintain this normal or near-normal performance with retention delays of up to 60s. In the spatial alternation tasks the only clear impairments came from those animals in the MDibo group in which the lesion had extended into the anterior thalamic nuclei; the degree of impairment correlating quite clearly with the extent of anterior thalamic damage. The only deficit that might be linked to damage in MD was found in the MDrf animals when they were

required to perform the task under conditions of high proactive interference, i.e. with both massed trials and retention delay.

The results of the present study strongly suggest that damage to fibres of passage made in the course of MD surgeries does not contribute to the effects of the lesions upon non-spatial working memory. This conclusion comes from the lack of difference between the MDrf and MDibo groups on the non-spatial (Y-maze) DNMS tasks, and also from the fact that when both groups were combined the extent of the DNMS acquisition impairment was still correlated with the extent of cellular damage in MD. These results lessen the likelihood that other studies of the effects of MDlesions have been compromised by damage to fibres of passage, and are therefore consistent with the prevalent view that any contribution of MD damage to diencephalic amnesia is due to the role played by the nucleus itself. However, the decline in the performance of the MDrf animals on the spatial working memory task under massed conditions (Experiment 4) does pose the question as to whethera difference between the MDrf and SHAM groups might have been demonstrated if the task difficulty had been further increased. If this were to be the case, this could be a possible explanation for some of the apparently contradictory findings of deficits in animals with non-chemical MD lesions (Brito et al, 1982; Means et al,

1974; Vicedomini et al, 1982) where the ability of rats to perform similar tasks of spatial working memory is taxed to an extreme degree. However, this hypothesis would have to await further experimentation in which rats with lesions made by either method could be compared on equally difficult spatial tasks to those used in these three studies.

Central to the results from the delayed non-matching to sample task was the finding that rats with MD damage were impaired on acquisition of the task, but not on its performance over delays. This differs from the results of previous experiments using exactly the same apparatus and procedure in which removal of neither the hippocampus (Aggleton et al, 1986), amygdala (Aggleton, Blindt, & Rawlins, 1989), fornix nor mammillary bodies (Aggleton, Hunt, & Shaw, 1990) disrupts the rate of acquisition of this task. Indeed, unpublished evidence that fornical lesions increase the rate of DNMS acquisition but severely impair the spatial alternation task points to a double dissociation with the effects of MD damage. These findings underline the very different contributions of the hippocampus and MD to learning and memory.

At first sight it may be tempting to draw parallels with the finding that MD lesions in monkeys can also impair acquisition of DNMS tasks (Aggleton & Mishkin, 1983b; Zola-Morgan & Squire, 1985). It should, however, be noted that this similarity may only be superficial, as the deficit

in monkeys is thought to be a consequence of the delay of 8 to 10 seconds between sample presentation and test (Overman, Ormsby, & Mishkin, 1990). No such delay occurred in the present task. Following acquisition of the task, monkeys with MD lesions show performance deficits with retention delays of 60 seconds (Aggleton & Mishkin, 1983b; Zola-Morgan & Squire, 1985), while those animals in the present study that were able to master the DNMS task performed at a near-normal level with the same retention delay. This apparent difference is, of course, biased by the exclusion of those rats that failed to master the task, and, had they been tested on the delay conditions after the limit of 400 training trials, there is little doubt that a clear group difference would have emerged.

One interpretation of results is that the effects of the MD lesions on DNMS tasks reflect a procedural learning deficit. While this is contrary to findings from monkeys (Aggleton & Mishkin, 1983a; Zola-Morgan & Squire, 1985), it is consistent with findings from other studies with rats. It has been shown that rats with MD lesions are very impaired at acquiring a reversal learning-set for olfactory discriminations (Slotnick & Kaneko, 1981). Similarly, it has been found that MD lesions can severely impair the acquisition of an odour discrimination-set (Staubli et al, 1987), although with extensive training this deficit could be partially overcome. This latter finding is reminiscent of

those animals in the present study that could perform the Y-maze task at normal levels once they had eventually mastered the task. Other acquisition deficits following MD lesions have been found using a compound visual-tactile discrimination task (Waring & Means, 1976; Weiss & Means, 1980), a go/no-go alternation task (Winocur, 1985), and a temporal alternation task in a straight alley (Beracochea et al, 1989). This array of results strongly suggests that MD lesions disrupt rule-learning on a wide range of tasks, adding weight to the idea of a procedural learning deficit. This may, however,be restricted to non-spatial tasksand notinconsistent with evidence that procedural learning for some tasks is preserved in Korsakoff's syndrome (Cohen & Squire, 1980).

It is possible that an apparent effect such as a procedural learning deficit may, in fact, be caused by some other aspect of the rats' behaviour which has been altered in some way by the lesion. In this case it might be that the MD rats were failing by perseverating in their choice of body-turn direction. In fact the analysis of correction trials per error over the first 100 trials, as reported in the results of Experiment 1, shows that this was not the case.

Similarly, it is possible that olfactory cues have an effect on the DNMS Y-maze tasks. Although the possibility that rats can 'cheat' to solve the Y-maze task by following

unintentionally provided olfactory cues was ruled out in previous, related studies (Aggleton et al, 1986; Huston & Aggleton, 1987), the control experiment described in the Appendix was run to test the rats on the same Y-maze task, but using cues which differed in the visual mode only. This was designed to show whether, when deprived of differential olfactory cues, the control rats would be equally impaired in learning as the MD rats. Unfortunately, neither lesion nor control animals showed any sign of learning the task at all, presumably due to the general reduction in salience, visual and olfactory, incurred by providing the limited range of cues available when furnishing the start/goal boxes with only one material. Despite this failure, the experiment served to confirm that the rats do indeed need the cues in the start/goal boxes to perform what is therefore clearly a task of recognition memory, and are not solving the task by, for example, following scent trails or accidental cues given by the tester. Other studies which are cited in the Appendix discussion do seem to indicate that the possible olfactory impairments of MD rats would not affect their performance in these experiments.

In conclusion then, it appears that MD is a structure of the brain which has been strongly implicated in memory functions. The evidence for this has come largely from studies of human cases of amnesia, which is necessarily slow and difficult to obtain in any degree of precision, and

is therefore often open to misinterpretation. Animal studies on the other hand can be controlled to a much greater degree, but have so far failed to show any clear picture of MD's involvement in memory functioning. Given that the nucleus has strong anatomical connections with other areas of the brain which are known to be involved in learning and memory, this study attempted to apply tests of working memory which are known to be sensitive to the amnesic effects of other brain lesions to rats with lesions in MD. The results showed that MD lesions had little if any effect on working memory, but reduced the rats' learning ability on the non-spatial test of working memory.

APPENDIX

NON-SPATIAL WORKING MEMORY - MONOCHROME BOXES

This experiment was designed to examine whether rats could learn the delayed non-matching to sample task without using olfactory cues, and to determine whether, under these conditions, there was a difference in the ability to learn the task between a lesion and a non-lesion group. The purpose of this was to rule out the possibility that rats with lesions in MD might show an impairment due not to a memory deficit, but to a sensory one, as the central division of MD is known to receive olfactory inputs (Cornwall & Phillipson, 1988, Groenewegen, 1988).

Method - The experiment was run in exactly the same way as the normal DNMS experiment described in Chapter One, with the exception that none of the pairs of start/goal boxes contained objects, and their inside surfaces were covered with monochrome photographic paper on which patterns had been printed which were designed to make each pair of boxes appear as distinctive as possible from all other pairs.

The subjects were 7 rats as described in the "General Methods" section of Chapter Two, randomly allocated

to two surgical groups (MDibo n = 4, SHAM n = 3). Ibotenic acid lesions and sham surgeries were carried out as described in "General Methods".

All other procedures were carried out as described in section 2.1, "General Methods".

Results - The experiment was abandoned after 250 trials as the animals were showing no significant evidence of learning the task, or of any lesion effect or interaction. Figure 16 shows that their scores were at chance and that they did not improve throughout the experiment.

Discussion - The animals in both groups could not learn the DNMS task when it was run with monochrome boxes, even though in all other respects the procedure was identical to Experiment 1. The conclusion then is that boxes distinguished only by monochrome patterns and without objects do not provide sufficient cues to permit recognition by rats. This may well be because the rats do, in fact, use olfaction to help recognise the different materials in the boxes. It may also be due to the lack of objects to provide sufficient three-dimensional visual information, or to a combination of the two.

Although this experiment helps to confirm that rats do, in fact, perform this task by recognition and not by following scent trails, it still fails to answer the

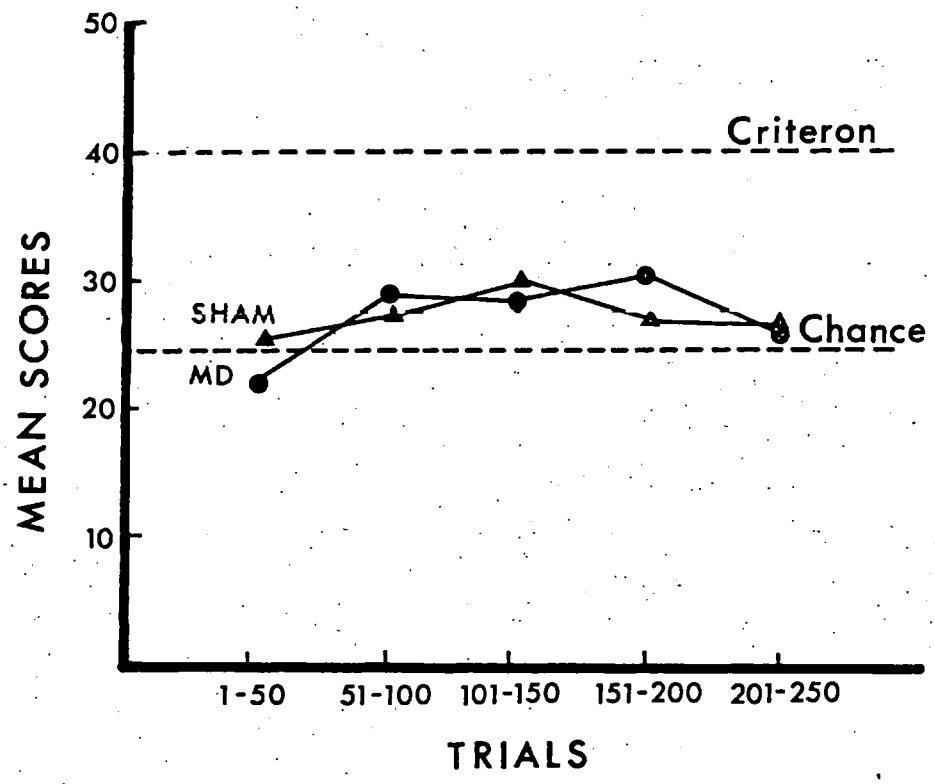

Fig. 16. Y-maze non-matching to sample using monochrome start/goal boxes: Trials 1 to 250.

question as to whether they use olfactory cues in the recognition of the boxes, and therefore whether the rats with MD lesions were at a possible disadvantage. While control tests in related studies have shown that rats do not rely on olfactory cues in order to perform the DNMS task (Aggleton, Hunt, & Rawlins, 1986, Huston & Aggleton, 1987), it is possible that such cues add to the salience of the different pairs of boxes.This explanation of the MD deficit is not, however, consistent with the findings that MD lesions do not affect detection thresholds (Eichenbaum, Shedlack, & Eckmann, 1980, Slotnick & Kaneko, 1981), and even though olfactory discrimination impairments have been reported (Eichenbaum et al, 1980; Slotnick & Kaneko, 1981; Staubli et al, 1987), these have been interpreted as indicating a procedural learning deficit rather than the consequence of a specific olfactory detection deficit (Staubli et al, 1987).

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